THE EFFECTS OF DIFFERENT SELENIUM SOURCES ON THE MEAT QUALITY AND BIOAVAILABILITY OF SELENIUM IN LAMBS

by
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Thesis presented in fulfilment of the requirements for the degree of Master of Science in Agriculture in the Faculty of AgriSciences at Stellenbosch University

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Co-supervisor: Prof CW Cruywagen

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Declaration

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Date: December 2012

Abstract

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In many parts of the world, soil is depleted of selenium (Se), leading to selenium-poor plants, animals and, therefore, humans. It was recognised that a study to examine the functionality of new products on the market to address this problem was required.

The purpose of this research were threefold: to compare the effects of sodium selenite (NaSe) and organically bound selenium sources on small ruminant performance, to investigate the bioavailability of these Se sources, and analyse their influence on carcass characteristics, meat quality and antioxidant capabilities. Fourty growing Döhne Merino wethers from the Southern Cape region, a selenium-deficient area, were used for the study. The animals were all fed the same basal diet in the adaptation period and were then allocated to one of four treatment groups: Control (CT), inorganic selenium (IS), organically bound Se A (OSA) or B: (OSB). The period of supplementation was 90 days.

This first study assessed the effect of the different Se sources on growth and Se bioavailability in the wethers. The wethers and the feed they consumed were regularly weighed to determine their growth and feed conversion rate (FCR) in the trial period. To gauge their Se level, blood samples were collected via jugular venipuncture at monthly intervals. The wool around the jugular was shorn and samples were collected on day 0 and day 90 for comparative Se level analysis. Liver, skeletal muscle and kidney samples were collected at day 90, directly after slaughter, to determine the Se level in these tissues.

No effect could be reported in the growth and FCR of the wethers between the supplementation groups. For whole blood Se levels there was an effect in the early part of the study, with a greater increase in Se levels for the organically bound Se groups, but in the end no effect on whole blood levels could be seen between the different Se treatments. Neither could any difference between the inorganic Se and organic bound Se treatments be found in the liver – however, the total Se concentration of the wool, kidney and

meat samples was greater in those animals offered organically bound Se when compared with those receiving a comparable dose of inorganic Se.

The second study evaluated the antioxidant capabilities of the different Se supplements in the wethers. Blood samples were taken monthly for plasma collection to test for Glutathione peroxidase (GSH-Px) activity and total antioxidative capacity (TAC) with the oxygen radical absorbance capacity (ORAC) assay. Liver, skeletal muscle and kidney samples were collected at day 90, immediately after slaughter and measured for GSH-Px activity.

With TAC, there was a significant effect for the treatment period between day 0 and day 90, however the treatments did not show any significant difference. No significant differences could be established between the different Se treatments for the GSH-Px analysis in any of the tissues. For the mean plasma values of the treatments no significant differences can be reported, but a significant difference was observed at day 30 in the contrast between the organically bound Se and the other treatment groups.

The third study was to evaluate the quality and lipid oxidation of muscle from those wethers supplemented with different Se sources. Skeletal muscle samples were collected at day 90, directly after slaughter to determine this. No differences in the meat quality of the wethers could be detected between Se sources after the 90-day supplementation period. Lipid oxidation was measured by determining TBA reactive substances (TBARS) and once again no differences could be detected.

Based on the results found in this investigation, it may be inferred that organically bound Se (OSA & OSB) supplementation will hold a number of advantages for small ruminants over inorganic Se supplementation. Animals fed the organically bound Se had reached adequate Se levels sooner on the organically bounded treatments than the inorganically bounded treated animals. The greater bioavailability of organically bounded Se over inorganic Se was proven by the increased Se levels in certain tissues and organs. Additionally, only the organically bounded Se could find a pathway to the wool, confirming that it was carried in an organic form (probably selenomethionine) in the body. Organically bound Se will therefore have a positive impact on small ruminant health and production, which will result in an indirect advantage for consumer health.

Opsomming

EFFEK VAN DIE SELENIUM BRON OP DIE BIOBESKIKBAARHEID VAN SELENIUM EN VLEIS KWALITEIT VAN LAMMERS

deur

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Die grond in groot dele van die wêreld word selenium-arm en dit lei na selenium-arm plante, diere en mense. Dit is waargeneem dat ‘n studie wat kyk na die funksionaliteit van nuwe produkte op die mark om die probleem aan te spreek nodig is.

Die doelwit van die studie was om verskillende selenium (Se) bronne te vergelyk en die uitwerking daarvan op klein herkouer prestasie te evalueer. Daar is gekyk na die biobeskikbaarheid, invloed daarvan op die karkas eienskappe en antioksidant vermoëns van die verskillende Se bronne. Veertig groeiende Dohne Merino-hamels van die Suid-Kaap-streek, 'n Se arm gebied is gebruik vir die studie. Die diere is almal dieselfde basale dieet gevoer in die aanpassing periode en dan toegeken aan een van vier behandelings: kontrole (CT), anorganiese Se (IS), organies gebinde Se A (OSA) of B: (OSB). Die tydperk van die aanvulling was 90 dae.

In die eerste studie is gekyk na die effek van die verskillende bronne van Se op die groei en die biobeskikbaarheid daarvan aan die hamels. Die hamels en voer verbruik, is gereeld geweeg sodat hul groei en voer omset verhouding (VOV) in die proef tydperk te bepaal. Bloedmonsters is versamel deur middel van die jugulêre venipuncture vir die Se vlak bepaling daarvan. Lewer, skeletspier en nier monsters is versamel op dag 90, direk na die slagting vir die Se vlak bepaling. Die wol rondom die nekslagaar is geskeer en monsters is versamel op dag 0 en 90 vir Se vlak analise.

Geen effek kan gerapporteer word vir die groei en VOV van die hamels tydens die aanvullings periode nie. Vir die bloed Se vlakke was daar 'n uitwerking in die vroeë deel van die studie, met 'n vinniger toename in Se vlakke vir die organies gebinde Se groepe, maar aan die einde kon geen effek gesien word tussen die verskillende Se behandelings nie. Geen verskil tussen die NaSe en organiese gebonde Se behandelings kon gevind word in die lewer nie. Die totale Se konsentrasie van die wol-, nier-en vleis

monsters groter was in die diere wat organies gebinde Se ontvang het wanneer dit vergelyk word met die wat 'n soortgelyke dosis van IS ontvang het.

Die tweede studie het die antioksidant vermoëns van die verskillende Se aanvullings in die hamels geëvalueer. Bloedmonsters is maandeliks geneem om plasma in te samel en te toets vir die glutathione peroksidase (GSH-Px) aktiwiteit en die totale anti-oksidant kapasiteit (TAC) met die suurstof radikale absorbansie kapasiteit (ORAC) toets. Lewer, skeletspier en nier monsters is versamel op dag 90, direk nadat die hamels geslag is. Glutathione peroksidase aktiwiteit is gemeet in die plasma, lewer, spier en niere. Daarbenewens is die TAC van die plasma ontleed, deur gebruik te maak van die ORAC toets.

Met TAC, was daar 'n effek vir die behandelings tydperk tussen dag 0 en dag 90, maar geen beduidende verskille tussen die behandelings nie. Geen beduidende verskille kon tussen die verskillende Se behandelings vir die GSH-Px analise in enige van die weefsel gevind word nie. Vir die gemiddelde plasma-waardes van die behandelings was daar geen beduidende verskille om te rapporteer nie, maar 'n beduidende verskil is met die kontraste tussen die organies gebinde en die ander behandelings waargeneem op dag 30.

Die derde studie was om die gehalte en lipied oksidasie van die spiere van hamels wat met verskillende Se bronne aangevul is, te evalueer. Skeletspier monsters is versamel op dag 90, direk nadat die diere geslag is om die gehalte daarvan bepaal. Geen verskille tussen Se bronne kon opgespoor word in die vleis gehalte van die hamels na die aanvullings tydperk van 90 dae nie. Lipied oksidasie is gemeet deur die bepaling van TBA reaktiewe stowwe (TBARS) en geen verskille kon opgespoor word nie.

Gebaseer op die resultate wat verkry is in hierdie ondersoek, kan dit afgelei word dat organies gebinde Se (OSA & OSB) aanvullings 'n aantal voordele sal inhou vir klein herkouers in verhouding tot die anorganiese Se aanvulling. Organies gebinde Se het 'n beter biobeskikbaarheid as NaSe want dit is beter geabsorbeer en geassimileer in die liggaam proteïen. Dit sal dus 'n positiewe impak op klein herkouer gesondheid en produksie hê, wat sal lei tot 'n indirekte voordeel vir die gesondheid van die mens.

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CHAPTER 1

General introduction

In world hunger, the most significant deficiency is protein-energy malnutrition. This is the shortage of sufficient protein (from meat and other sources) and other foods that produce energy, measured in calories, found in all of the basic food groups (World hunger, 2011).

New technologies and better farming systems are required to meet the growing demand for protein. South Africa is no different from the rest of the world and the soil is becoming depleted of Se, leading to selenium-poor plants, animals and therefore humans. Selenium is recognised as an essential trace element for the maintenance of health, growth and a myriad of biochemical-physiological functions. In recent years the importance of adequate Se levels to maintain human and animal health has become more evident.

In South Africa, thousands of people are directly dependent on sheep farming for their food and livelihood, with millions dependent in turn on livestock farmers to provide them with sufficient good quality protein. The world population is growing, with more mouths to feed and shrinking land resources suitable for livestock production. Therefore, with ‘food security’ the new buzz-word, new technology and better farming systems are required to meet this demand.

The specific purpose of this research were threefold: to compare the effects of inorganic and organically bound Se sources on small ruminant performance, to investigate the bioavailability of these Se sources, and analyse their influence on carcass characteristics, meat quality and antioxidant capabilities.

Chapter 2

Literature review

1. Selenium

In 1817 selenium (Se) was discovered in the flue dust of iron pyrite burners by the Swedish chemist, Jons Jacob Berzelius (Levander, 1986; Sunde, 1997). Since its discovery Se has had an interesting history. In the 1930s Se was identified as a toxic agent implicated in alkali disease and blind staggers (Franke, 1934; Franke & Potter, 1935; Moxon, 1937) and in 1943, Nelson *et al.* classified Se as a carcinogen. It was considered a dangerous element until 1957 when Schwarz and Foltz identified Se to be one of three compounds that prevented liver necrosis in rats, thus establishing Se as a nutritionally essential trace mineral. Nutritionists and scientists then started numerous studies to discover the metabolic function of the element and record the consequences of its deficiency in human and animal diets. It was not until 1974 that Se was added as a supplement to animal diets.

The discovery of severe Se deficiency in certain parts of China in the 1970s has proven that this trace element is also an essential nutrient for human health (Keshan Disease Research Group, 1979; Whanger, 1989; Levander, 1991; Ge & Yang, 1993), and its role has been reviewed recently (Rayman, 2000, 2004). It was reported (Phipps *et al*., 2008) that between 1975 and 1995, Se intake in the United Kingdom decreased from around 60 to 34 µg/d per person, which means that the current (2011) intake is about half of the UK Reference Nutrient Intake. The Se content of foods obtained from plants and animals are, to a great extent, influenced by the availability of soil Se for uptake by plants (Shrift, 1969). Evidence suggests that the Se intake in large parts of Europe is too low when compared to the recommended intake (Rayman, 1997; Rayman, 2000).

The decreasing Se intake in the last decades has been mainly credited to a change in the source of wheat for bread and cereal products, from primarily North American to European origin (from a high to low selenium content). These are reflected in decreasing levels of Se in human plasma and serum (Biesalski, 2005). This decline has caused concern because suboptimal intake is associated with a number of serious health issues.

Two diseases have been associated with severe endemic Se deficiency in humans: a juvenile cardiomyopathy (Keshan disease), and a chondrodystrophy (Kaschin-Beck disease). Each occurs in rural areas of China and Russia in food systems with exceedingly low Se supplies. Keshan disease has been noted in mountainous areas where the soil Se levels are very low (Combs, 2001). In these areas humans have shown the lowest reported Se levels. Dramatic reductions in Keshan disease incidence have been achieved by the use of oral Sodium selenite or selenite-fortified table salt (Keshan Disease Res. Group, 1979). Low blood Se levels have been measured in patients with several other diseases (Combs, 2001).

Children with protein deficiency diseases, Kwashiorkor or marasmus, tend to be low in Se as Se occurs in food proteins.

In nature Se occurs inorganically as selenite, selenate, elemental Se or selenide, and organically bounded forms include selenomethionine (SeMet) and selenocysteine (SeCys) (Ike *et al*., 2000). A number of positive effects were observed in feeding trials, when researchers increased the Se dosage for the animals. The US Government gave approval for the supplementation of Se to the diets of animals in 1979, while they regulated both the concentration (0.1 ppm) and the source (sodium selenite or selenate) of supplemental selenium. In 1987 the regulation was modified and the allowed supplemental Se level was increased to 0.3 ppm in ruminant diets, but the approved sources (sodium selenite and selenate) did not change. With constant research and new data, the FDA (2003) had to update the regulation in September 2003 to permit the use of organically bound Se in the form of Se yeast in the diets of beef and dairy livestock. The maximum supplementation rate allowed in the US was maintained at 0.3 ppm of Se, though it was higher at 0.5ppm in Europe.

2. Absorption and metabolism

Selenium absorption in the intestine is affected by the form of dietary Se (Sunde, 1997). A number of studies done on various animal species including sheep, pigs (Wright & Bell, 1966) and rats (Whanger *et al.*, 1976) confirmed that the duodenum is the site were the greater part of dietary Se is absorbed, regardless of source. Selenium that occurs naturally in feeds is largely found as selenoamino acids, with selenomethionine (SeMet) compromising more than 50 % of total Se in many feed ingredients, it fulfils the criteria of an essential aminoacid (Schrauzer, 2003). Inorganic selenium is generally supplemented in animal diets as sodium selenite. Sodium selenite is absorbed through the small intestine by simple diffusion, while SeMet is actively absorbed by the same amino-acid transport system as methionine (Sunde, 1997). Both forms of Se are well absorbed in monogastric animals. Overall, however, the absorption of Se is poorer in ruminants and this may be connected to the reduction of dietary Se to insoluble forms in the rumen environment (Spears, 2003). The absorption of Se is not regulated by dietary Se concentration or Se status, and Se homeostasis is primarily regulated by the urinary excretion of Se (Schlegel *et al*., 2008).

The chemical form and the amount of Se ingested will regulate the metabolism thereof. Following absorption, sodium selenite and SeMet are metabolised differently (Sunde, 1997). Sodium selenite is reduced to selenide which can be used for synthesis of selenocysteine (SeCys), or methylated and excreted in urine. Selenocysteine is the form of Se present in selenoenzymes such as Glutathione peroxidase (GSH-Px). SeMet can be incorporated into proteins in place of methionine, or be reformed to SeCys. Dietary methionine levels will affect the extent to which SeMet is incorporated into general proteins (Butler *et al.,* 1989). The pathway of the metabolism of NaSe was summarised by Sunde (1997). First, the selenate is converted to selenite (Axley & Stadtman., 1989); this is then nonenzymically reduced

by glutathione to elemental Se, forming seleno-diglutathione (Ganther, 1966). With the lack of oxygen, selenide is formed by glutathione reductase from seleno-diglutathione (Hsieh & Ganther, 1975); from where it can take varies routes. The selenide can be methylated to various forms (Hsieh & Ganther, 1977), but the relevant path is where selenide bind to selenium-binding proteins. It can also form part of the synthesis of selenoproteins (Sunde, 1997) by tRNA, which will convert the inorganic Se to its organically bound form, which is found in mammalian tissues.

According to Sunde (1997); organically bound Se are metabolised in a different way than NaSe. This Se can easily be integrated into a protein such as selenomethionine (SeMet), (Hoffman *et al.,* 1970; McConnell & Hoffman, 1972) this can then be metabolised to Se-adenosyl methionine, and then further to Se-adenosyl homocysteine (SeAH; Markham *et al.,* 1980). The SeAH can then be converted to selenocysteine (SeCys), which can then be then be degraded. The degrading process will release selenite, or differently be degraded to elemental Se, which can be reduced further to selenide (Esaki *et al.,* 1982). The metabolism of the SeMet can follow another route as describe by Steele & Benevenga in 1979, where the SeMet is transaminated to methaneselenol. The methaneselenol can then be further converted into selenide, (Sunde, 1997) from where the metabolism will follow the route as described above.

3. Bioavailability

Bioavailability may be defined as that part of Se absorbed from the gastrointestinal tract which is metabolically available for the maintenance of the normal structures and physiological processes of an organism under defined conditions (Wolffram, 1999). The bioavailability of organically bound trace minerals in ruminants is proven to be superior to that of inorganic sources (Kincaid *et al.,* 1997; Spears, 2003). Criteria that have been used to assess Se bioavailability include GSH-Px activity (Gabrielsen & Opstvedt, 1980), tissue Se concentrations (Osman & Latshaw, 1976), and prevention of Se deficiency symptoms (Cantor *et al*., 1975a, b). Bioavailability estimates for Se sources (especially SeMet relative to selenite) varies greatly depending on the criterion used. Feeding SeMet or selenised yeast increases Se concentrations in blood (Ortman & Pehrson, 1999) and muscle compared with selenite (Osman & Latshaw, 1976; Mahan *et al*., 1999). Glutathione peroxidase activity is the preferred criterion for assessing Se bioavailability and measures the utilisation of Se in animals fed on selenium-deficient diets. The activity of GSH-Px in plasma, red blood cells, and a number of organs responds in a dose manner to dietary Se concentrations which fall below requirement (Oh *et al*., 1976). Clearly Se incorporation into non-specific proteins does not represent utilisation of Se for a specific biochemical function. When chicks were fed selenium-deficient diets after receiving supplemental Se from either selenite or SeMet, whole blood (Moksnes & Norheim, 1986) and plasma GSH-Px (Payne & Southern, 2005) declined more rapidly in birds which had originally received selenite. This confirms that SeMet from non-specific proteins is released during normal protein catabolism and used as a source of Se for GSH-Px synthesis.

4. Selenium deficiency

Deficiencies of Se have been observed in cattle and sheep under grazing conditions worldwide. These deficiency symptoms include white muscle disease (Muth *et al*., 1958), particularly in young animals or lambs born to selenium-deficient ewes, loss of Glutathione peroxidase activity and selenoprotein (Yeh *et al*., 1997), suppression of immunity (Yamini & Mullaney, 1985) and infertility in ewes grazing in selenium-poor pastures. The economic losses of poor performance and wool growth due to marginal Se deficiency may be underestimated because of the absence of clinical signs (Hill *et al.,* 1969).

Likely responses to supplementation can be expected in growth, wool growth and fertility in sheep with selenium-poor grazing or diets (<0.1 mg Se/kg DM). Van Ryssen *et al.* (1989) observed that the greatest effects of inorganic Se versus high-selenium wheat on Se concentration in tissues were to be found in the liver, muscle and wool. Clinical deficiency symptoms are however not readily observed; Van Ryssen and co-workers (1999) recognised lambs with Se concentrations of between 9 and 26ng Se/ml whole blood as selenium-deficient, although clinical deficiency symptoms had not been observed. Puls (1994) regarded levels of < 50ug/L as indicative of Se deficiency in sheep. However, Se levels regarded as deficient, marginally deficient and adequate differ slightly between sources.

Inorganic Se supplementation is still the norm to prevent Se deficiency in ruminant animals, but evidence is now emerging that the organic form has additional benefits over inorganic Se supplementation of livestock feeds. According to Mahan, (1999) inorganic Se has a lower bioavailability in the rumen and some of the consumed Se is utilised by microorganisms for their metabolism and only small amounts is incorporated into body proteins (Wolfram, 1999).

Organicly bound Se on the other hand can by-pass the rumen, as it is in the form of selenoamino acids. Selenomethionine is found naturally in edible plant protein and is actively transported through intestinal membranes during absorption and actively accumulated in the liver and muscle (Lyons *et al*., 2007). Those different characteristics make commercially available organically bound Se supplements a suitable form of Se for animal nutritional supplementation.

5. Selenium concentrations in tissue

The concentration of Se that can be found in the body tissues is dependent on a number of factors. The chemical form, the length of time over which it was consumed, the amount of Se provided by the diet and the species of animal, will all have an influence. Although Se is present in all tissues, an especially high concentration is found in the liver, kidney, and spleen, and to a lesser extend, skeletal muscle, cardiac muscle, intestine, and lung. Tissue concentration of Se is influenced by amount and chemical form of Se in the diet (Pond *et al*., 1995). About 45% of total body Se is associated with the muscle, 4.6% with the liver and 6.9% with the kidneys (Grace, 1985).

In young animals, Se concentration can also depend on the level of dietary Se consumed by the dam. When NaSe is fed to a young subject, the tissue concentration approaches a plateau as the Se level in the diet rises. The effect is not the same when SeMet is the Se source; the Se concentration keeps on rising to some threshold beyond that of selenite. Latshaw (1975) reported that the Se concentration of chicken liver and muscle was doubled by feeding Se in natural feedstuffs as opposed to feeding the same level of NaSe. The result was not the same when measuring the blood Se concentration of chickens which were fed SeMet, compared to an equivalent amount of selenite. Cantor *et al.* (1982) reported that SeMet greatly increased Se concentrations in the pancreas, muscle, and gizzard but not in the liver when compared to selenite.

6. Selenium toxicity

Originally the importance of Se in animal health was related to its toxic properties when it was proven that it causes malformation in animals and in extreme situation can lead to death (Moxon, 1937; Meyer & Buran, 1995), and certain plants such as the *Astragalus* species in the USA were found to accumulate selenium. Livestock grazing on these plants was poisoned, a condition called alkali disease (Thacker, 1961). The signs of acute Se toxicity in ruminants include elevated temperature and pulse rate, watery diarrhoea, extensive tissue haemorrhage and oedema. Death is due to circulatory failure and myocardial damage (Howell, 1983). Chronic Se toxicity occurs when sheep consume plants for a period of time which contain >3ppm Se and it is associated with loss of appetite, lameness, poor growth and wool production, delayed conception and blindness (Howell, 1983). In 2006, Tiwary concluded that the organically bound Se source, SeMet is slightly less toxic than the inorganic Se source, NaSe.

7. Selenoproteins

The physiological roles of Se began with ground-breaking work by Rotruck *et al.* (1973) which identified Se as a stoichiometric component of Glutathione peroxidase. Soon thereafter in the mid-1980s, more selenoproteins were discovered and selenium biochemistry began to broaden. Selenium has now been identified as an important part of more than 30 selenoproteins (Sunde, 1997; Arthur, 2000).

7.1 Glutathione peroxidase

The processes of oxidation and reduction are part of the body's biochemistry and as respiration happen, a by-product known as peroxides are produced. These peroxides can produce free radicals, which can be destructive to the body as it could damage or destroy cells (Arthur, 2000). However, a group of enzymes, known as Glutathione peroxidases (GSH-Px), are in place to defend the body against these harmful peroxides (Arthur, 2000).

In 1957, Mills was the first to communicate the actions of GSH-Px, this was followed by Rotruck *et al*. in early 1973 that implied that Se formed an important part of GSH-Px, this was confirmed later that year by Flohe *et al*. (1973). The metabolic function of GSH-Px is imperative for cells, as it forms part of the

process that is in charge of the metabolism and the detoxification of oxygen. The best known biochemical role for Se is as part of the active site of the enzyme GSH-Px, as it helps to prevent oxidative damage to body tissues (Hoekstra, 1974) and DNA (Combs & Clark, 1985).

Ursini *et al.* (1995) described four structurally and genetically different forms of selenium-containing GSH-Px that exist in different tissues or parts of the cell. However, in excess of thirty selenoproteins have been identified, including several forms of GSH-Px and other tissue-specific selenoproteins with antioxidant activity (Behne *et al*., 1994).

7.2 Other selenoproteins

Selenoproteins that have also been identified are Iodothyronine deiodinases (ID), selenoprotein P, selenoprotein W, thioredoxin reductase, selenium binding proteins, sperm capsule selenoprotein and a protein in the epithelial cells of the rat prostate. The ID group is after GSH-Px the largest group of selenoproteins, and is further divided into ID 1; ID 2 and ID 3. The main function of ID is performed around the actions of the thyroid hormone and Se forms an integral part of this (Kohrle *et al*., 2000).

Selenoprotein P was first described by Hill *et al*. (1991), but at this point, its function in the body is still unclear. The cause of lambs suffering from white muscle disease was identified by Pederson *et al*. (1972) to be a missing selenoprotein; it was later recognised by Vendeland *et al*. (1995) to be muscle selenoprotein W. The selenoprotein thioredoxin reductase is involved in the regulation of disulphide groups within enzymes and transcription factors (Sun *et al*., 1999). The sperm capsule selenoprotein forms a major part of the sperm capsule, which is consistent with the role of Se in maintaining normal fertility (Venzina *et al*., 1996).

The diversity of these identified selenoproteins emphasises the wide range of biochemical pathways and thus physiological functions that can be caused by changes in Se status of the animal. Thus characterisation of ‘newer’ selenoproteins may identify clinical problems that have not been linked to Se deficiency.

8. Immunology

Selenium deficiency has been reported to decrease both cellular and humoral immune function in man and laboratory animals (Combs & Combs, 1986). The knowledge of specific mechanisms in livestock is less detailed than in laboratory animals although the increase in susceptibility to disease in deficient livestock is well documented (Maas, 1998). Sordillo *et al*. (1997) reported that Se deficiency is an established risk factor in mastitis incidence and has been correlated with decreased bactericidal activity of neutrophils and the severity of mastitis infection. Injections of barium selenite decreased the incidence of mastitis in dairy goats (Sanchez *et al*., 2007), and Se yeast in the diet has decreased episodes of

diarrhoea in calves (Guyot *et al*., 2007). Marginal Se depletion has lowered the resistance of chickens to the protozoan parasite *Eimeria tenella* (Colnago *et al*., 1994).

9. Oxidation

Most animals, plants, and microorganisms depend on oxygen for the efficient production of energy. However, free radicals derived from oxygen can damage many types of biological molecules and is potentially toxic for living organisms. The formation of free radicals is a pathobiochemical mechanism involved in the initiation or progression of various diseases (Hogg, 1998). The presence of natural antioxidants in living organisms enables their survival in an oxygen-rich environment (Halliwell, 1994). In livestock production, free radical generation and lipid peroxidation are responsible for the development of various diseases, reduction in animal productivity, and product quality (Hurley & Doane, 1989; Weiss, 1998; McDowell, 2000).

There are several methods that exist to measure total antioxidant capacity, but the majority of literature refers to three methods;

1. FRAP (ferric reducing ability of plasma – Benzie,1996)
2. TEAC (trolox equivalent antioxidant capacity – Rice-Evans,1994)
3. ORAC (oxygen radical absorbance capacity – Cao,1999)

Accordind to Cao (1998) is the ORAC method of measuring antioxidant status the most accepted, because its measurements are based on fluorescence rather than absorbance. The ORAC test is a hydrogen atom transfer assay that determines antioxidant capacity by measuring competitive kinetics. It consists of three basic components: a fluorescent probe, a radical donor and a fixed amount of antioxidant against which to compare the sample antioxidant capacity. As the radical donor increases, the fixed amount of fluorescent agent present in the reaction mixture will progressively become quenched. Any antioxidant present in the system would scavenge the radicals, effectively out-competing the fluorescent probe as substrate (Cao *et al*., 1993). It is the only methodology that links the inhibition time with the degree of inhibition (Ou *et al*., 2001), thus increasing the sensitivity and so permits a lower molar ratio of antioxidant sample to reagents, thus minimising the possibility of cross-reactions between the two.

A variety of different stress conditions are associated with the over-production of free radicals and thus cause a disturbance in the prooxidant/antioxidant balance, leading to potential tissue damage (Jaeschke, 1995). Stress conditions are usually grouped into: nutritional, environmental, and internal stress of which all will stimulate the generation of free radicals. Once free radical production exceeds the antioxidant system's capacity to neutralise it, lipid peroxidation causes damage to unsaturated lipids in cell membranes, amino acids in proteins, and nucleotides in DNA, resulting in membrane and cell integrity disruption. This inevitably will result in decreased productive and reproductive performance (Dalton *et al*., 1999).

10. Selenium in meat

Selenium plays an important role in muscle (meat), not only to increase Se availability for human consumption through food, but also to improve meat quality. Meat colour, fat content, in pack purge and price determine how consumers perceive quality, which in turn influences purchasing behaviour (Grunert, 1997; 2006). Meat colour is the foremost selection criterion used by consumers in the purchase of meat and is commonly used as an indicator of freshness. Cooked meat colour, juiciness and tenderness are also important product quality cues during consumption. Consumers regard meat tenderness as the most important palatability trait (Pietisik & Shand, 2004) and juicy meat is generally preferred over dry meat (Risvik, 1994).

According to various trials, the majority of the physical properties of meat described above (i.e., colour, texture, and firmness of raw meat; juiciness and tenderness of cooked meat) will be to some extent be dependent on the meat's water-holding capacity (WHC) (DeVore *et al*., 1983; Avanzo *et al.,* 2001; Lawrie, 1998). Although some of these trials are confounded by the inclusion of other components such as Vitamin C and E (Munoz *et al.,* 1996; Torrent, 1996). Mahan *et al.* (1999) reported no difference for drip loss in pig meat with NaSe addition, and a linear increase in Hunter L value (paleness) of muscle also with added selenite. There are a number of trials looking at drip loss in broiler meat, and some suggest a positive effect of Se-yeast over NaSe, but overall the evidence is inconclusive (Edens, 1996; Naylor *et al.,* 2000). Clyburn *et al*. (2000) suggested a trend for beef flavour and flavour intensity to be improved by organically bounded Se, although the data reported were somewhat inconclusive.

11. Selenium in wool

Wool is composed of a complex protein named keratin, and is built up from different amino acids (D'Arcy, 1990). With a number of trials on the influence of amino acids on wool growth, Reis & Schinckel (1963), Reis *et al.* (1967, 1979 and 1990) pointed out that the amino acid, methionine play a major role in wool production. Selenomethionine (as described earlier) are a product from the metabolism of the two sources of Se supplementation as pointed out by Edens (2002). We come to expect the results from Wilkins & Kilgour, (1982), Hill *et al.* (1969) and Langlands *et al.* (1991a, 1991b) which established that wool is very sensitive to selenium deficiency and that Se supplementation significantly increased wool production.

However, Wright & Bell, (1966) and Kincaid *et al.* (1997) found that the absorption and metabolism of these two sources are different, especially in ruminants, because of the microorganisms in the rumen and it was Spears (2003) who concluded that the bioavailability of organic trace minerals is superior to inorganic sources in ruminants. Consequently, we have to hypothesise that the organic Se source, SeMet, which will be used in the current study will have a greater deposition in the wool fibres than will the inorganic source. This will confirm the results from Davis *et al.* (2008) and Van Ryssen *et al.* (1989)

who found that the wool from sheep supplemented with organically bound Se sources had significantly higher Se levels than those supplemented with inorganic sources.

12. Organically bound v. inorganic Se

For many years it has been recognised that the selenoamino acids SeMet and SeCys are the sources of naturally occurring Se (Burk, 1976; Levander, 1986; Cai *et al*., 1995) and constitute 50-80% of the total Se in plants and grains (Butler & Peterson, 1967). Selenomethionine cannot be directly synthesised from selenite or selenate by animals (Cummins & Martin, 1967; Sunde, 1990).

The tissue retention of organically bound or inorganic Se differs (Ku *et al.,* 1973). Inorganic Se has a reduced bioavailability in the ruminant because of the anaerobic conditions in the rumen. Although part of the oxidised form of Se (Sodium selenite) is reduced in the rumen to the unabsorbable elemental or inorganic selenide forms, which is not absorbed through the rumen or the intestinal tract, some of the consumed NaSe is used by rumen microbes for their metabolism. The microbial protein thus formed with Se can pass into the small intestine and serve as a source of dietary Se for the ruminant. The selenium-enriched yeast protein is hydrolysed in the rumen and small intestine to the respective amino acids. The selenoamino acid, SeMet can be non-specifically incorporated into body protein (Kincaid, 1995) and most probably serve as Se storage capacity. Subsequent research has demonstrated that blood GSH-Px activity in ruminants is lower when the inorganic form of the element is fed to dairy animals, but that Se levels in milk can be increased up to four or five times by feeding the lactating cows organically bound Se (Pehrson *et al.*, 1999).

13. OSA and OSB

In this study two organically bound Se sources produced from whole cell yeasts were investigated, along with Sodium selenite. The first organically bound Se source was OSA (organically bound selenium A) and is an inactivated whole cell yeast product containing elevated levels of Se. OSA contains 2000ppm of total Se, the major part in its natural food form, L(+) selenomethionine. It is produced by growing yeast, *Saccharomyces cerevisae,* in the presence of measured amounts of Se. Live yeast cells absorb the Se and biochemically transform it into selenomethionine and other selenoproteins (Lallemand, 2007). The second source investigates; OSB (organically bound selenium B) differs from OSA in the application of fermentation method and the resulting amino-acid profile. It contains similar levels of total Se as well as selenomethionine as OSA.

Several studies conducted in collaboration with different research partners have demonstrated the main effects of OSA in meat type and fattening ruminants. It has a higher bioavailability, producing increased selenium levels in blood and tissue, with increased GSH-Px activity in the blood (antioxidant seleno-dependant enzyme). A decrease in muscular problems and the occurrence of myopathies (white muscle disease) in young animals was recorded along with an recovery of the meat quality, which became less

exudative. Organic selenium OSA increases this phenomenon owing to its active transportation through the intestinal gut, compared to the passive way for the inorganic forms (Lallemand, 2007).

In this study, two organically bound Se sources (labelled OSA and OSB) were compared with each other and NaSe supplementation, or no Se supplementation (Control) for its effects on the performance of lambs, the effect of the supplementation on tissue and plasma Se levels and anti-oxidant status of the animals and finally the effect thereof on meat characteristics of lambs.

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CHAPTER 3

General materials and methods

Abstract

This study aimed to determine the effects of dietary supplementation of Döhne Merino wethers with different selenium (Se) sources on various measurable parameters. Fourty growing Döhne Merino wethers from the Southern Cape region of South Africa, a selenium-deficient area, were used for the study. The animals were all fed the same basal diet in the adaptation period and were then allocated at random to one of four dietary treatment groups: Control (CT), containing Se from the basal diet only; the inorganically bounded group, of basal diet with added Sodium selenite (IS); or one of two groups fed organically bounded Se in the basal diet with added organically bound Se A (OSA) or B: (OSB). The period of supplementation was 90 days.

Introduction

Selenium is recognised as an essential trace element and its deficiency in ruminants can result in numerous deficiency symptoms. According to Edens (2002) there are two sources of Se with which to supplement animal diets, an inorganic source and an organically bound source. Inorganic Se is available mostly in the form of Sodium selenite (NaSe), while organically bound Se is most common as selenised yeast in the form of selenomethionine. According to Mahan (1999) NaSe has a lower bioavailability in the rumen and some of the consumed Se is utilised by microorganisms for their metabolism. Organically bound Se instead can by-pass the rumen as it is in the form of selenoamino acids.

OSA is an inactivated whole cell yeast product containing elevated levels of Se. OSA contains 2000ppm of total Se, the major part in its natural food form, L(+)selenomethionine. It is produced by growing yeast, *Saccharomyces cerevisiae*, in the presence of measured amounts of Se. Live yeast cells absorb Se and biochemically transform it into SeMet and other selenoproteins. Selenomethionine is naturally found in edible plant protein and is highly bioavailable. Those different characteristics make OSA the most suitable form of Se for animal nutritional supplementation. OSB differs from OSA in fermentation method and the resulting amino-acid profile, but it contains the same levels of total Se as well as SeMet as OSA.

Expected likely responses to supplementation with OSA can include increases in tissue and blood Se content, increases in the GSH-Px (antioxidant selenodependant enzyme) activity in the blood due to the higher bioavailability of OSA, a decrease in muscular problems and myopathies (white muscle disease) in young animals, and an improvement in meat quality. The aim of this study was to establish the

advantages of supplementing Döhne Merino wethers with OSA and OSB rather than the norm of Sodium selenite (IS).

Materials and methods

The study was carried out at Stellenbosch University's experimental farm, Welgevallen, Stellenbosch, South Africa. The project was approved and conducted under the ethical clearance of Subcommittee B of the Research Committee of Stellenbosch University; reference number: 2007B03006

Animals and feeds

Forty Döhne Merino wethers, each with an average initial body weight of 40.7 kg, were purchased from the Bredasdorp area in the Southern Cape, a selenium-deficient area. The Döhne Merino is a well-balanced dual-purpose sheep breed that allows the producer to market a quality, heavyweight lamb and fine-medium white wool. It has established itself as one of the leading woollen breeds in South Africa, and its percentage of the national flock is rising. There are a number of advantages synonymous with the versatility of the Döhne Merino, including hardiness, adaptability and less selective grazing habits (which minimise management and production costs). Their high fertility and rapid lamb growth, heavy carcasses with low fat distribution, excellent feed conversion makes them ideal to finish on good pastures or in the feedlot, and of course the production of high-quality wool. Overall they give an added stability to the economy of woollen sheep farming (Döhne Merino Breed Society of SA).

The wethers were randomly allocated into individual pens (1m x 2m) in an enclosed but adequately ventilated shed with a wooden slatted floor. The animals had free access to drinking water. In the pre-trial period the lambs were adapted to a selenium-poor diet (Table 3.1), which also served as the control diet during the trial, until sufficiently low blood selenium concentrations were reached.

Table 3.1: Formulation of the basal diet indicating the calculated selenium concentrations

Feedstuff	**% Inclusion (As is)**	**Se mg/kg**	**Se inclusion**
Wheat straw	76.00	0.11	0.08
Maize starch	15.00	0.01	0.00
Molasses meal	5.00	0.00	0.01
Urea	1.00	0.00	0.00
Premix Control	3.00	0.85	0.02
Total	100		**0.11 mg/kg (0.14)***

* Value in bracket indicates the actual analysed selenium concentration as opposed to the calculated concentration based on the formulation of the feed from the individual ingredient's Se content

The basal diet consisted mainly of wheat straw, corn starch as an energy source and urea, with the addition of Mutton Gainer (NuTec (Pty) Ltd., Pietermaritzburg, South Africa) (Table 3.3) containing the various treatments (CT, IS, OSA or OSB). The raw materials were analysed individually for Se levels; these values were used to formulate a basal diet with a calculated Se value of 0.11mg/kg. The complete final basal diet was also analysed with the true Se concentration in the basal diet at 0.14mg/kg. Vitamin E was allowed at the level of 312.5 IU/kg as part of the Mutton Gainer, and included in 3% of the diet. Whole blood Se concentration was monitored until the concentration was in the range of 80-100ng/ml, indicating a marginal Se deficiency (Koller *et al*., 1983). This marked the onset of the trial and the lambs were randomly grouped and assigned to one of four dietary treatments (Table 3.2): Control (CT, 0.14mg/kg Se), containing Se from the basal diet only; the inorganic group, of basal diet with added Sodium selenite (IS, 0.36mg/kg Se); or one of two groups fed organically bound selenium (Se) in the basal diet, with added organically bound Se A (OSA, 0.32mg/kg Se) or B: (OSB, 0.36mg/kg Se).

Selenium-poor raw materials were sourced for the basal diet, all were individually analysed for Se levels, according to which the feed was formulated. The wheat straw was finely chopped in a hammer mill and was hand-mixed with other raw materials for the trial with the specially prepared premixes from NuTec (Pty) Ltd., Pietermaritzburg, South Africa (Table 3.3) – which had been supplemented with different sources of selenium to reach a supplemental selenium level of +/- 0.27mg/kg (Table 3.2).

Table 3.2: Physical and chemical composition of treatment feeds (Control (CT), Inorganic Se (IS), Organic Se B (OSB), Organic Se A (OSA)) indicating selenium concentration of the experimental diets fed to the Döhne Merino wethers

	CT	**IS**	**OSB**	**OSA**
	Raw material composition, As fed basis			
Wheat straw g/kg	760	760	760	760
Maize starch g/kg	150	150	150	150
Molasses meal g/kg	50	50	50	50
Urea g/kg	10	10	10	10
Premix CT g/kg	30	0	0	0
Premix IS g/kg	0	30	0	0
Premix OSB g/kg	0	0	30	0
Premix OSA g/kg	0	0	0	30
	Chemical Composition, DM basis			
Moisture g/kg	95.9	100.9	104.3	105.8
Ash g/kg	50.2	63.7	55.0	70.6
Crude fat g/kg	6.5	6.9	7.0	6.9
Crude fibre g/kg	395.8	370.9	315.8	341.2
Crude protein g/kg	84.7	110.7	93.1	125.0
N g/kg	13.6	16.2	14.9	17.3
Se mg/kg	0.11 (0.14)*	0.27 (0.36)*	0.26 (0.36)*	0.27 (0.32)*

* Values in brackets indicates the actual value as per analysis as opposed to the calculated concentration based on the formulation of the feed from the individual ingredient's Se content

All the final feed mixes were analysed to get the actual Se levels in the feed, and these values differed slightly from the formulated values. This could be attributed to ineffective hand-mixing with a high percentage of wheat straw in the diet.

Table 3.3: Chemical composition of Mutton Gainer 125 as per specification of NuTec (Pty) Ltd., (Pietermaritzburg, South Africa) adapted for for inclusion in the Control (CT), inorganic selenium (IS), organically bounded selenium A (OSA) or B (OSB) dietary treatments

	CT	**IS**	**OSB**	**OSA**
	g/kg	**g/kg**	**g/kg**	**g/kg**
Protein (min)	1250	1250	1250	1250
% from NPN	100	100	100	100
Urea (max)	354.7	354.7	354.7	354.7
Fibre (max)	50	50	50	50
Moisture (max)	120	120	120	120
Calcium (max)	140	140	140	140
Phosphorus (min)	2.3	2.3	2.3	2.3
Magnesium (min)	7.33	7.33	7.33	7.33
Sulphur (min)	19.88	19.88	19.88	19.88
	mg/kg	**mg/kg**	**mg/kg**	**mg/kg**
Cobalt	7.82	7.82	7.82	7.82
Copper	193.75	193.75	193.75	193.75
Iodine	7.82	7.82	7.82	7.82
Iron	843.76	843.76	843.76	843.76
Maganese	562.5	562.5	562.5	562.5
Selenium CT	0	0	0	0
Selenium IS	0	6.25	0	0
Selenium OSB	0	0	6.25	0
Selenium OSA	0	0	0	6.25
Zinc	562.5	562.5	562.5	562.5
Vitamin B1	125	125	125	125
Niacin	3155	3155	3155	3155
Salinomycin	625.03	625.03	625.03	625.03
Zinc Bacitracin	625.03	625.03	625.03	625.03
	IU/kg	**IU/kg**	**IU/kg**	**IU/kg**
Vitamin A	125 000	125 000	125 000	125 000
Vitamin D3	31 250	31 250	31 250	31 250
Vitamin E	312.5	312.5	312.5	312.5
Mass	**16 kg**	**16 kg**	**16 kg**	**16 kg**

Chemical analysis was performed on the four experimental diets by the Animal Science Laboratory, Stellenbosch University, South Africa, for Moisture, Ash, Crude Protein, Crude Fibre, Ether extract and Nitrogen. Ash was determined according to the official method of AOAC International (2002), method 942.05, Moisture according to the AOAC (2002) official method 934.01, Crude Fibre according to the AOAC (2002) official method 962.09, and Fat (crude) or Ether extract according to the AOAC (2002) official method 920.39. Nitrogen was determined on a Leco-FP528 according to the official method of AOAC International (2002). The chemical composition of the diets is presented in Table 3.2.

Sampling

Feed samples were collected throughout the trial of all treatments, and finely milled and sealed. Blood samples were collected at monthly intervals via jugular venipuncture with an 18-gauge needle into a 6ml vacutainer (K3E K_3EDTA) and packed in ice. Liver, muscle and kidney samples were collected after slaughter by stunning and exsanguination sealed separately and stored on ice. The wool around the jugular was shorn and samples were collected on day 0 and 90, individually packed and sealed.

The sheep were slaughtered at a commercial abattoir using standard South African techniques. Treatment (transport, handling, etc.) was similar for all groups. After being electrically stunned (4 Ampere, 200 Volts for 4 seconds) the sheep were exsanguinated and dressed. No electrical stimulation was applied.

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CHAPTER 4

The effect of different sources of selenium supplementation on the growth and selenium deposition in the whole blood, tissue and wool of Döhne Merino wethers

Abstract

This study aimed to determine the effect of different selenium (Se) source supplementation on the growth, whole blood Se levels, tissues and wool of Döhne Merino wethers, which are marginally deficient in Se. Forty growing Döhne Merino wethers from the Southern Cape region, a selenium-deficient area were used for the study. The animals were all fed the same basal diet containing marginal levels of Se in the adaptation period and were then randomly allocated to one of four dietary treatment groups once sufficiently low Se levels were reached: Control (CT), containing Se from the basal diet only; the inorganic group, basal diet with added Sodium selenite (IS); or one of two groups fed organically bound selenium (Se) in the basal diet with added organically bound Se A (OSA) or B: (OSB). The period of supplementation was set at 90 days, wherein the wethers, and the feed consumed, were regularly weighed to determine their growth and feed conversion rate (FCR) in the trial period. Blood samples were collected via jugular venipuncture for Se level determination at monthly intervals. Liver, skeletal muscle and kidney samples were collected at day 90 after slaughter by stunning and exsanguination for Se level determination. The wool around the jugular was shorn and samples were collected on days 0 and 90 for Se level analysis.

No effect was observed on the growth and FCR of the wethers across the supplementation groups. However, there was a greater increase in Se levels for the organically bound Se groups in the early part of the study. At the end of the trial, no effect from treatment could be seen across the different Se sources supplemented in the basal diets. Results from the liver analysis were in contrast to findings of previous researchers, and no difference between the inorganic and organically bound Se treatments could be found. However, the total Se concentration of the wool, kidney and meat samples was greater in those animals offered organically bound Se when compared with those receiving a comparable dose of Se selenite. This confirmed our hypothesis that organic Se sources have a greater bioavailability in small ruminants and are more readily available than that of inorganic sources. Therefore, organically bound Se supplementation rather than inorganic supplementation can have advantages for animal and human health.

Key words: selenium bioavailability, wethers, Sodium selenite, organically bound selenium

Introduction

Selenium is recognised as an essential trace element and its deficiency in ruminants results in white muscle disease (Muth *et al.,* 1958), loss of glutathione peroxidase activity (Rotruck *et al.,* 1973), reduced selenoprotein W content in the muscle which can possibly have an influence on the incidence of white muscle disease (Yeh *et al.,* 1997), suppression of immunity (Yamini & Mullaney, 1985), and impaired growth and fertility (Weiss *et al.*, 1990). Because feed grown in many areas of the world, including the Southern Cape region of South Africa (Van Ryssen, 2001) is deficient in Se, its supplementation is often necessary to prevent the potential clinical signs of Se deficiency in livestock grazing these areas (Stevens *et al.,* 1985). There are two forms of Se available to supplement livestock diets with (Edens, 2002), an inorganic mineral salt, such as NaSe, or an organic form such as Se-enriched yeast. The absorption and metabolism of these two sources can be different, especially in ruminants, because of the microorganisms in the rumen which partly convert the inorganic Se compounds to insoluble elemental Se or selenide, which cannot be absorbed in the lower intestinal tract (Butler & Peterson, 1961; Wright & Bell, 1966). Another limitation with inorganic Se supplementation is the apparent short duration of Se storage in the animal (Surai, 2006a, b). Selenium from organic sources can however be absorbed through amino acid transport, and directly incorporated into body protein, improving its absorption and retention (Peter *et* al., 1982). There is a higher deposition of Se in the tissues of animals when the organically bound form of Se is supplemented compared to inorganic Se supplementation (Mahan & Parrett, 1996; Kim & Mahan, 2001a, b). Selenium reserves can therefore be build-up in the body with organically bound Se, which can then be utilised when increased levels of Se is required under stress conditions (Rock *et al.,* 2001). Both forms of Se appear to be used in the body to construct specific selenoproteins, but according to Rayman *et* al. (2008) Se enters cellular metabolism at different points, depending on its chemical form.

The objective of this research was to compare the effect of specific organically bound Se sources, against that of inorganic Se looking at the growth in sheep and the depositing thereof into the blood, body tissues (liver, muscle, kidney) and wool.

Materials and methods

The reader is referred to Chapter 3 for the description of the experimental procedures and the treatments used in determining the effect of the different Se supplementations on the performance of the lambs. Table 3.2 is included again for the benefit of the reader regarding the different treatments used in the study.

Table 3.2: Physical and chemical composition of the control (CT), inorganic selenium (IS), organically bounded selenium A (OSA) or B (OSB) diets, indicating the selenium concentration of the experimental diets fed to the Döhne Merino wethers

	CT	IS	OSB	OSA
	Physical composition, As fed basis			
Wheat straw g/kg	760	760	760	760
Maize starch g/kg	150	150	150	150
Molasses meal	50	50	50	50
Urea g/kg	10	10	10	10
Premix CT g/kg	30	0	0	0
Premix IS g/kg	0	30	0	0
Premix OSB g/kg	0	0	30	0
Premix OSA g/kg	0	0	0	30
	Chemical Composition, DM basis			
Moisture g/kg	95.9	100.9	104.3	105.8
Ash g/kg	50.2	63.7	55	70.6
Crude fat g/kg	6.5	6.9	7	6.9
Crude fibre g/kg	395.8	370.9	315.8	341.2
Crude protein g/kg	84.7	110.7	93.1	125
N g/kg	13.6	16.2	14.9	17.3
Se mg/kg	0.08 (0.14)*	0.27 (0.36)*	0.26 (0.36)*	0.27 (0.32)*

* Values in brackets indicates the actual value as per analysis

Performance characteristics

Animal growth performance was measured by weighing the wethers individually at the start off the trial (day 0), day 30, day 60 and day 90, just before they were slaughtered. Average daily gain (ADG) was calculated from these values. All four groups received 1.2kg of their specific complete mixed feed every day, 600g in the morning and 600g in the afternoon. Daily feed intake was calculated by weighing the previous day's leftover feed, before feeding again. The feed conversion ratio (FCR) of the wethers was calculated by dividing the total feed consumed by the lambs in each group by their total weight gain over the experimental period (feed consumed (kg) per kg gain).

Sample preparation

The wool around the jugular was shorn to clear the vein for easy blood collection. These wool samples from the jugular were collected on days 0 and 90 of the trial, individually packed and sealed for subsequent Se analysis. Blood samples were collected on a monthly basis via jugular venipuncture with an 18-gauge needle into a 6ml vacutainer (K3E K_3EDTA) and packed in ice. Liver, muscle and kidney samples were collected after slaughter by stunning and exsanguination separately sealed and stored in ice. All the samples were taken to the Western Cape Provincial Veterinary Laboratory, Stellenbosch, South Africa for Se analysis immediately after collection.

Chemical analysis

All Se analysis (whole blood, liver, muscle, wool and kidney) was done by the Western Cape Provincial Veterinary Laboratory, Stellenbosch, South Africa; employing the fluorometric method of Koh & Benson (1983) to determine the Se levels in all the provided samples. One ml of the blood samples and 0.1g of the liver, muscle, wool and kidney samples were measured and placed in a digestion tube. Four blank tubes consisting of 1ml of 0.1M HNO_3 and four tubes for each standard containing 1ml of standard were also prepared with the samples. Four ml of acid mixture were added and placed in the digestion block which is housed in the wash-down fume cupboard.

The temperature controller was set to heat at 120^{o}C for 1 hour, then to heat at 160^{o}C for 6 hours and maintained at 120^{o}C for 30 hours. Tubes were removed and allowed to cool for a few minutes, 1ml 1:1 HCl was added to the tubes and placed in the digestion block at 120^{o}C for 30 minutes. Tubes were removed and allowed to cool for a few minutes. Fifteen millilitres EDTA solution and 1ml DAN solution and then 5ml cyclohexane were added. Tubes were then placed in a shaker for 1 minute and then in a 60^{o}C water bath for 40 minutes. The warm water was replaced with tap water and tubes were cooled down for 5 minutes. It was shaken again and left to stand for 30 minutes. The fluorescence of the supernatant cyclohexane was measured by aspirating it directly into the fluorometer (Perkin-Elmer, Selton, USA.). The Se content of the blood was reported as ng/ml (ppb) and that of the tissue and wool samples as mg/kg (ppm).

Statistical analysis

Repeated measures ANOVA were made of treatments over a number of days to measure whether interactions between treatments and days were significant, and multiple comparisons were made of the interaction effects. Appropriate contrasts were investigated among treatments. If interactions were not significant, the main effects were similarly analysed. When ANOVAs were done on the interested response variables among the treatments, the treatment effects were analysed with Bonferroni multiple comparisons (if significant) with appropriate contrast among the treatment effects (Statistica version 8.1 (2008)). Differences were considered as significant when $P<0.05$.

Results and discussion

The wethers' growth performance, final body weight, weight gain, average daily gain (ADG), feed intake and feed conversion ratio (FCR) were not significantly influenced by the dietary supplementation of different Se sources (Table 4.1). The control (CT) group received 0.14mg/kg DM (Table 3.2) of Se which was present in the basal feed raw materials. The results, combined with the fact that the lambs had reach maturity indicate that the Se content of the CT diet may have satisfied the Se requirements of the group.

Growth rate is not likely to be influenced by Se supplementation, unless there is a clear shortage of the mineral (Johansson *et al.,* 1990). No significant results on growth rate, feed intake or feed to gain ratio could be found by Juniper *et al.* (2006), in a 112 day feeding trial, where growing lambs were either supplemented with an organically bound Se source or an inorganic Se source. Additionally, no influence could be reported in a trial on growing calves by Skřivanová *et al.* (2007), were they looked at different levels and source of Se and the influence thereof on the performance of the calves. The physical performance of finishing beef steers was left unaffected by Se source and the concentration thereof (Lawler *et al.,* 2004).

Table 4.1: Growth performance data of wethers supplemented with different selenium sources in their diets. Mean ± standard error of the mean

Variable	CT	IS	OSB	OSA
N	10	10	10	10
Initial body weight (kg)	39.85 ± 0.93	39.65 ± 0.98	40.55 ± 0.71	41.45 ± 0.79
Final body weight (kg)	43.65 ± 0.70	42.6 ± 0.83	43.95 ± 0.67	44.05 ± 0.99
Body weight gain (kg)	3.8	2.95	3.4	2.6
Average daily gain (g) (ADG)	46	36	41.5	31
Daily feed consumption (kg)	1.03	1.04	1.03	1.05
Feed conversion ratio (FCR, kg feed per kg gain)	22.4	28.8	24.8	33.8

No significant differences were found between treatments for any of the parameters

With Se supplementation to sheep (Hartley & Grant, 1961) and young cattle (Wichtel *et al.*, 1996) positive effects could only be reported in cases where exceptionally low levels of Se were present. However, in studies with beef cattle and dairy cows no effect could be seen on the weight gain when the marginally and normal concentration diets were supplemented with Se (Weiss *et al.,* 1983 & Gunter *et al.*, 2003).

In the present study, the Se status of the wethers at the start of the trial was classified as marginally deficient accoring to Se concentrations reported by Puls (1994). Background Se present in the compositional feedstuffs may also have been sufficient to meet the basic Se requirements of the wethers. This can possibly explain the lack of difference in growth performance between the various trial groups additionally to the animals already having reached puberty.

Whole blood was collected on a monthly basis and analysed immediately afterwards by the Western Cape Veterinary Laboratory, Stellenbosch, South Africa, and the values received are displayed in Table 4.2. After the Se depletion period, and although the wethers were randomly assigned to the treatments, the OSB group had significantly (P=0.017) lower Se levels than the rest at the start of the trial. This must be brought into the equation throughout the rest of the trial when conclusions are made. On day 30 the OSA group had significantly (P=0.037) higher Se levels than the CT group, with the OSB group having

the biggest increase in average Se levels from day 0. However, the large variance in data observed is likely the explanation for a lack in significant differences (0.059) between the OSA and IS treatments. Bringing into account the low starting value of the OSB group and the low P value of the OSA group, the organically bound Se treatments had a definite tendency to create higher Se levels than the IS and CT groups at day 30.

Table 4.2: Whole blood selenium levels measured in Dönhe Merino wethers supplemented with different selenium sources. Mean ± standard error of the mean

	Treatment				
Parameter	CT(ng/ml)	IS(ng/ml)	OSB(ng/ml)	OSA(ng/ml)	P-value
Day 0	102.0^{a} ± 8.59	95.0^{ab} ± 5.78	86.11^{b} ± 10.89	98.67^{ab} ± 12.94	0.02
Day 30	100.8^{a} ± 6.58	100.88^{ab} ± 9.63	111.0^{ab} ± 13.51	113.7^{b} ± 8.51	0.04
Day 60	199.11^{a} ± 13.89	195.1^{a} ± 19.42	212.0^{a} ± 9.26	213.6^{a} ± 16.88	0.07
Day 90	129.78^{a} ± 7.17	134.75^{a} ± 10.74	136.63^{a} ± 10.32	137.89^{a} ± 12.09	0.37

^{ab} Means in the same row with different superscripts differ (P<0.05)

This result is consistent with previous reports by Juniper *et al.* (2008) and Nicholson *et al.* (1991) in beef cattle, Gunter *et al.* (2003), Ortman & Pehrson (1997, 1999) in dairy cows and Van Ryssen *et al.* (1989), Qin *et al*. (2007), Davis *et al.* (2008) in sheep. All reported greater whole blood Se concentrations in animals supplemented with organically bound Se than those offered diets with comparable amounts of IS Sodium selenite as the source of supplementary Se. Therefore, it is concluded that organically bound Se can readily improve an animal's Se status and is superior to IS in this regard.

However, on day 60 of the trial there was a great increase across all of the groups in whole blood Se levels, with no significant differences that could be detected between treatments. It is evident therefore that IS can result in an improved Se status of the animals, and to the same extent as an organically bound Se source, but that IS requires a longer period of supplementation to attain this level.

This result corresponds to findings of Petrera *et al*. (2009) in goats, Pehrson *et al.* (1999) in cows and Nicholson *et al.* (1993), Awadeh *et al.* (1998) in beef cattle, who reported no overall difference in whole blood Se when the diets were supplemented with an organically bound Se or inorganic Se source.

Looking at the whole picture it is evident that the two organically bound Se sources were the quickest to improve the whole blood Se levels of the Döhne Merino wethers in the first 30 days of the trial. Adequate whole blood Se levels were reached at this point by the organically bound Se sources and the inorganic Se source could catch up. This led to no detectable differences between treatments at the end of the trial, which resulted in the conclusion that IS can result in an improved Se status to the same extent as an organically bound Se source, but that IS requires a longer period of supplementation to attain this level.

The Se levels of the muscle, liver and kidney samples that were collected after slaughter are presented in Table 4.3. These results are consistent with the reports by Taylor (2005), Juniper *et al.* (2008) and Combs & Combs (1986) which indicated that the Se concentrations ranked the highest in the kidney, followed by the liver and the skeletal muscle. However, in diets of extremely high Se concentration, the liver can have higher Se concentrations than the kidney in lambs (Cristaldi *et al.,* 2004).

Table 4.3: Selenium levels of muscle, liver and kidney samples of wethers supplemented with different selenium sources measured on a fresh tissue basis. Mean ± standard error of the mean

	Treatment				
Parameter	CT(mg/kg)	IS(mg/kg)	OSB(mg/kg)	OSA(mg/kg)	P-Value
Muscle	0.538^{a} ±0.084	0.535^{a} ±0.059	0.756^{b} ±0.074	0.809^{b} ±0.182	< 0.01
Kidney	4.11^{a} ±0.82	4.4^{a} ±0.66	4.63^{ab} ±0.55	5.4^{b} ±0.71	< 0.01
Liver	1.55^{a} ±0.069	2.71^{b} ±0.42	2.66^{b} ±0.362	2.96^{b} ±0.647	< 0.01

^{ab} Means in the same row with different superscripts vary (P≤0.05)

In the skeletal muscle, the two organically bound Se treatments resulted in significantly higher Se concentrations than the control and the inorganically supplemented treatments, with no difference between the control and the inorganic Se treatment. This is consistent with Ehlig *et al.* (1967), Van Ryssen *et al.* (1989) and Qin *et al.* (2007), who reported a greater incorporation of organically bound Se into skeletal muscle than that from inorganic sources.

The kidney tissue from the organically bound OSA group showed significantly higher Se levels than the inorganic selenium (IS) and that of the CT; these levels did not however differ significantly from the OSB supplement (P=0.0537). Similarly, Qin *et al.* (2007) and Juniper *et al.* (2008) have found that organically bound Se fed to ruminants resulted in higher amounts of Se in the kidney than did inorganically bound Se.

All three Se treatments resulted in significantly higher Se levels in the liver, but there were no differences between the inorganic and organic treatments. This is in contrast to previous research by Van Ryssen *et al*. (1989) and Weiss (2003), who indicated that, in addition to muscle, the liver is one of the organs where one often finds better effects of organically bound Se supplements. These researchers did however also report higher Se levels in the blood of the animals at the end of their studies, which differs from the current study. Once the Se is absorbed into the blood, regardless of its form, it will be absorbed by the liver where it will be utilised. The liver Se level is therefore not dependent on the supplemented form so much as on the Se levels present in the blood.

In 1980, White reported that Se concentrations in wool have a significant linear correlation with the Se intake. In the present study wool samples were collected from the Döhne Merino wethers at the start (day

0) and at the end (day 90) of the trial and sent to the laboratory for determination of the Se levels (Table 4.4). At day 0 after random allocation of the wethers into their four groups, the OSA group had significantly higher Se levels than the CT group. This difference was however put into perspective at day 90, when the OSA group had a threefold advantage over the CT group, and more than double the levels of the IS group. The OSB group has also outperformed the CT and IS groups significantly, with no difference present between CT and IS.

Table 4.4: Selenium levels of wool from wethers supplemented with different selenium sources measured at the beginning and end of the trial. Mean ± standard error of the mean

	Treatment				
Parameter	CT (ppm)	IS (ppm)	OSB (ppm)	OSA (ppm)	P-Value
Onset	0.358^{a} ±0.053	0.391^{ab} ±0.048	0.435^{ab} ±0.118	0.458^{b} ±0.074	0.02
Termination	0.43^{a} ±0.06	0.53^{a} ±0.09	0.95^{b} ±0.26	1.26^{c} ±0.27	< 0.01

^{abc} Means in the same row with different superscripts vary (P<0.05)

The outcome of this trial is consistent with Van Ryssen *et al.* (1989) and Davis *et al.* (2008) who both found significantly higher levels of Se in the wool of sheep that were fed with an organically bound selenium source than an inorganic source.

The contrast estimates were calculated (Table 4.5) for the Se concentration in the blood between the CT and the organic Se (OSA and B) groups, the CT and the IS, and for the IS and the OS (A and B) groups. All contrasts were calculated at day 30 as well as day 90. Significant results were found between the CT and OS (A and B), and between the IS and OS at day 30. This correlates with the previous findings of the benefit of OS over IS at day 30, in line with a number of researchers (as discussed earlier). The lack of any response at day 90 complements the findings of other researchers who reported no difference between IS and OS in whole blood.

The contrast estimates were also calculated for the Se concentration in the kidney, muscle, liver and wool between the CT and the IS group, the CT and OS groups and the IS and OS groups. Significant results were found across all calculations for the muscle, kidney and wool contrast estimates. Looking at the contrast estimates for whole blood at day 90 and the liver between IS and OS, no differences were observed. This strengthens the earlier argument that differences between Se sources will only appear in the liver if differences were observed in the blood.

Table 4.5: P-value contrast estimates between different selenium treatment groups for whole blood, tissue and wool samples

	P-values		
Whole blood	CT vs IS	CT vs OS	IS vs OS
Day 30	0.345	0.000*	0.007*
Day 90	0.208	0.982	0.162
Tissue & wool	CT vs SE	CT vs OS	IS vs OS
Muscle	0.000*	0.000*	0.000*
Kidney	0.009*	0.002*	0.022*
Liver	0.000*	0.000*	0.599
Wool	0.000*	0.000*	0.000*

* Means the contrast estimate is highly significant (P<0.01)

Conclusion

In recent years the importance of adequate Se levels to maintain human and animal health has become more evident. Since the acceptance of organically bound Se supplementation for animals, a number of studies have been performed on all species to establish the advantages thereof.

In the present study no advantage could be established for the supplementation of Se in any form to improve the growth and FCR of wethers. The reason for this could be because Se levels in the wethers were only marginally deficient and that there was some Se available to all groups from the feed, which may have been sufficient to meet the basic Se requirements of the wethers. Also, the wethers had relatively high BW and age at the onset of the experimental phase of the study, thereby decreasing their growth potential and therefore the potential to observe significant growth responses.

In the whole blood Se study, it was found that organically bound Se is more readily available in the first 30 days of the trial to the wethers to rectify their Se shortages than the inorganic source. At the end of the trial (day 90) no differences were observed between treatments. It is therefore evident that inorganic Se can also result in an improved Se status of the animals, and to the same extent as an organically bound Se source, but that inorganic Se requires a longer period of supplementation to attain this level.

With the tissue and wool analysis, results consistent with previous reports were obtained in the muscle, kidney and wool samples, with greater incorporation of organically bound Se into these tissues than that of inorganic sources. The organically bound Se was therefore proven to have a higher bioavailability for wethers than the inorganic Se source. This is mainly due to the different absorption paths of the two Se sources: unlike inorganic Se, the organically bound sources can be absorbed through amino-acid transport and directly incorporated into body protein (Hoffman *et al*., 1970). A possible advantage of this

is that the Se reserves which are built up in the body from the organically bound Se will be available in stress conditions when the Se requirement is increased. This however still needs further studies to confirm that this accumulation of selenium will be available to the animal in stress conditions. The Se accumulation in the body protein will also hold benefits for the consumers of these animal products, providing more available Se in their diets. Therefore, organically bound Se sources can be utilised in the production of functional food for human consumption.

Results for Se levels in the liver for the present study contradicts with previous research reports, showing no significant differences in Se levels between the inorganic and organically bound Se treatments. However, the whole blood Se levels found at the end of the trial of the current study also differed from these previous reports; suggesting that Se levels in the liver are probably be more dependent on the Se levels present in the blood of the animal than on the supplemented form of Se.

It is clear that organically bound Se has a higher bioavailability for small ruminants than NaSe. Organically bound Se will therefore have a positive impact on small ruminant health and production, which will result in an indirect advantage for human health.

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CHAPTER 5

The effect of different sources of selenium supplementation on the antioxidant status in the plasma and body tissues of Döhne Merino wethers

Abstract

The effects of different selenium (Se) supplements on the antioxidant status of small ruminants were studied in forty Dohne-Merino wethers purchased from the Southern Cape region of South Africa, a selenium-deficient area. The animals were all fed the same basal diet in the adaptation period and were then randomly allocated to one of four dietary treatment groups: Control (CT), containing Se from the basal diet only; the inorganic group, fed the basal diet with added Sodium selenite (IS); or one of two groups fed organically bound selenium (Se) in the basal diet with added organically bound Se A (OSA) or B (OSB). The period of supplementation was 90 days. Blood samples were taken monthly for plasma collection to test for Glutathione peroxidase (GSH-Px) activity and total antioxidative capacity (TAC) with the oxygen radical absorbance capacity (ORAC) assay. Liver, skeletal muscle and kidney samples were collected at day 90, after the wethers were slaughtered, and measured for GSH-Px activity.

There was a noticeable effect in TAC between day 0 and day 90 but the Se treatments did not differ significantly. No significant differences in the GSH-Px analysis in any of the tissues could be established between the different selenium treatments. For the mean plasma values of the treatments no significant differences could be reported, but a significant difference was observed at day 30 with a contrast between the organically bound Se and the other groups. It does appear that the organically bound Se is more readily available for the wethers to rectify any Se shortages and therefore increase the GSH-Px activity in the plasma. The supplementation of wethers with organically bound Se, rather than inorganic selenium, can have advantages for wethers in their recovery time from such ailments as white muscle disease.

Key words: antioxidant status, selenium, wethers, GSH-Px, ORAC

Introduction

Free radicals are produced by all animals as a by-product of metabolism; these can cause cell damage or even cell death (Weiss, 2005). But to prevent or counteract this damage nature produces various antioxidants, including superoxide dismutase (SOD), catalase and GSH-Px (Özturk-Ürek *et al*., 2001).

As early as 1957, Mills discovered GSH-Px and in 1973, Rotruck *et al*. identified Se as an integral part of the enzyme, six GSH-Px enzymes have been identified since (Sunde, 1997). The primary functions of the GSH-Px enzymes are to detoxify hydrogen peroxide (H_2O_2) and to convert lipid hydroperoxides to non-toxic alcohols (Jenkinson *et al*., 1982; Halliwell & Gutridge, 1989).

Antioxidants are molecules that can easily and harmlessly give up an electron. GSH-Px requires Se as a cofactor and contains a SeCys amino acid residue in the active site of each monomer that participates in the actual mechanism of the enzyme. The oxidation of NADPH to $NADP^+$ is accompanied by a decrease in absorbance at 340nm (A340). The rate of decrease in the A340 is directly proportional to the GSH-Px activity in the sample. One unit of GSH-Px is defined as the amount of enzyme that will cause the oxidation of 1 nmole of NADPH to $NADP^+$ per minute at 25ºC. Glutathione Peroxidase activity is therefore expressed as nmole/min/ml/ug protein = Units/ml (u/ml) (Stressgen, Assay Designs, Michigan, USA).

Considering the role that Se has in the GSH-Px enzymes and taking into account the function of these enzymes, we can hypothesise that different sources of Se will have an influence on plasma antioxidant capacity. The measure of antioxidant capacity considers the cumulative action of all the antioxidants present (vitamin A, E, C, superoxide dismutase, catalase, reduced glutathione and GSH-Px) (Pasupathi, *et al.,* 2009) in plasma and body fluids, thus providing an integrated parameter rather than the simple sum of measurable antioxidants (Ghiselli *et al*., 2000).

In 1996, Paszkowski & Clarke defined total antioxidant capacity (TAC) as a measure of overall free-radical scavenging potential. TAC is therefore the total ability of the body to protect itself from the destructive side-effects of physiological metabolism. Antioxidant capacity can be measured by means of several methods, such as trolox equivalent antioxidant capacity (TEAC) (Miller *et al.,* 1993), total radical-trapping antioxidant parameter (TRAP) (Ghiselli *et al.,* 1995), ferric reducing ability of plasma and biological antioxidant potential (FRAP & BAP) (Benzie & Strain, 1996), and the oxygen radical absorbance capacity (ORAC) assay (Cao *et al*., 1993). The ORAC assay is arguably the most accepted and accurate indicator of antioxidant capacity, mainly because it is based on measurements of fluorescence rather than absorbance (Cao & Prior, 1998). It is also the only assay that measures both inhibition time and degree of inhibition for an antioxidant (Huang *et al.,* 2002; Huang *et al*., 2005).

ORAC is a hydrogen atom transfer (HAT) assay that determines antioxidant capacity by measuring competitive kinetics. It consists of three basic components: a fluorescent probe, a radical donor and a fixed amount of antioxidant to compare the sample antioxidant capacity against a standard as described by Ou *et al*. (2001). Basically, as the radicals increase, the fixed amount of fluorescent agent present in the reaction mixture progressively becomes quenched. Any antioxidant present in the system would scavenge the radicals, effectively out-competing the fluorescent probe as substrate. This ultimately results in the extended viability of the fluorescent probe and would thus increase the area under the curve (AUC) generated. By measuring fluorescent intensities over time, a kinetic curve can be drawn from which the antioxidant capacity in any given sample can be deduced from the total AUC.

The aim of this study was to investigate the effect of supplementing Döhne Merino wethers with different sources of Se on their antioxidant status. Their total antioxidant capacity and GSH-Px activity in the plasma, muscle, kidney and liver were measured and used as indicators of their antioxidant status.

Materials and methods

The reader is referred to Chapter 3 for the description of the experimental procedures and the treatments used in determining the effect of the different Se supplementations on the performance of the lambs. Table 3.2 is included again for the benefit of the reader regarding the different treatments used in the study.

Table 3.2: Physical and chemical composition of treatment feeds indicating selenium concentration of the experimental diets fed to the Döhne Merino wethers

	CTR	IS	OSB	OSA
	Physical composition, As fed basis			
Wheat straw g/kg	760	760	760	760
Maize starch g/kg	150	150	150	150
Molasses meal g/kg	50	50	50	50
Urea g/kg	10	10	10	10
Premix CT g/kg	30	0	0	0
Premix IS g/kg	0	30	0	0
Premix OSB g/kg	0	0	30	0
Premix OSA g/kg	0	0	0	30
	Chemical Composition, DM basis			
Moisture g/kg	95.9	100.9	104.3	105.8
Ash g/kg	50.2	63.7	55	70.6
Crude fat g/kg	6.5	6.9	7	6.9
Crude fibre g/kg	395.8	370.9	315.8	341.2
Crude protein g/kg	84.7	110.7	93.1	125
N g/kg	13.6	16.2	14.9	17.3
Se mg/kg	0.08 (0.14)*	0.27 (0.36)*	0.26 (0.36)*	0.27 (0.32)*

* Values in brackets indicates the actual value as per analysis

Sampling

Blood samples were collected on a monthly basis via jugular venipuncture with an 18-gauge needle into an anticoagulant 6ml vacutainer (K3E K_3 EDTA) and packed in ice. These samples were taken to the laboratory, centrifuged at 1500 x g for 10-20 minutes at 4°C and the upper yellow plasma was collected with a pipet and stored at -80°C according to Stressgen, Assay Designs, Michigan, USA, for the GSH-Px and ORAC assays.

Liver, muscle and kidney samples were collected after slaughter by stunning and exsanguination. The tissue samples were perfused with 1 * PBS plus 0.16mg/mL heparin to remove blood components and clots. The tissues were then homogenised in 5-10ml/g of tissue 1 x cold assay buffer containing 0.4mM PMSF, other protease inhibitors, and 1% Triton X-100 (peroxide-free). The samples were centrifuged at 10,000 x g for 10-20 minutes at 4ºC to remove insoluble material, the supernatants were recovered into small aliquots, snap-frozen and stored at -80ºC according to Stressgen (Assay Designs, Michigan, USA) for analysis of the GSH-Px activity.

Chemical analysis

The ORAC procedure used an automated plate reader (FLx800 Fluorescence Microplate Reader) with 96-well plates. Analyses were conducted in phosphate buffer (PB) pH 7.4 at 37°C. Peroxyl radical was generated using 2.2'-Azobis (2-amidino-propane) dihydrochloride (AAPH) (153mM), which was prepared fresh for each run. Fluorescein ($8.16*10^{-5}$mM) was used as the substrate and Trolox (400µM) was used for standard curve. Plasma samples were defrosted at room temperature and diluted 200 x with distilled water. Twenty-five µl of PB, Trolox and sample were pipetted into their designated wells, 150µl fluorescein was added to every plate, and the plates were incubated for 10 minutes at 37°C. To each well, 25µl AAPH were added which initiated the reaction. Fluorescence was measured for 180 minutes at 1 minute intervals. Excitation wavelength = 485±20 nm; emission wavelength = 530±nm

A glutathione peroxidase activity kit was purchased from Stressgen, Catalog # 900-158, (Assay Designs, Michigan, USA) and the absorbance was calculated with an ELx800 Absorbance Microplate Reader at 25°C. The principle of the coupled enzymatic assay first described by Paglia & Valentine (1967) was applied. Glutathione (GSH), glutathione reductase (GR) and NADPH dissolved in phosphate buffer and sample were mixed in cuvettes. Then tert-butylhydroperoxide (BHPx) was added to initiate the reaction in a total volume of 200µL, immediately after the reaction was initiated, absorbance was measured at 340nm every minute over a 15 minute period. The protein concentrations of the samples were calculated by the method of Bradford, (1976) to express GSH-Px activity per unit of protein.

Statistical analysis

Repeated measures ANOVA were done on treatments over a number of days to measure if interactions between treatments and days were significant, and multiple comparisons were made on the interaction effects. Appropriate contrasts were investigated among treatments between successive days. If interactions were not significant, main effects were similarly analysed. When ANOVAs were done on the relevant response variable among the treatments, the treatment effects were analysed with Bonferroni multiple comparisons (if significant) with appropriate contrast among the treatment effects (Statistica version 8.1 (2008)). Differences were considered significant when $P<0.05$.

Results and discussion

Whole blood was taken on a monthly basis from the wethers and the plasma was collected from these blood samples in the laboratory. The ORAC assay was followed and the total antioxidant capacity (TAC) of the plasma was calculated, and is displayed in Table 5.1. No significant differences could be established between any of the treatments, suggesting that the level of naturally occurring selenium present in the feed raw materials may have satisfied the selenium requirements of the CT group. Large

variation was observed throughout the ORAC analysis and could possibly have played a role in the lack of significant results between the treatments.

Table 5.1: Total antioxidant capacity (TAC) of wether plasma with different selenium treatments. Mean ± standard error of the mean

	Treatment								
Parameter	CT(µmol/l)		IS(µmol/l)		OSB(µmol/l)		OSA(µmol/l)		P-Value
Day 0	5 228[a]	± 1 118	4 982[a]	± 1 581	4 526[a]	± 1 525	5 392[a]	± 2 021	0.53
Day 30	6 201	± 1 441	6 033	± 1 402	5 544	± 1 824	5 919	± 1 962	0.58
Day 60	7 582	± 2 447	7 180	± 2 984	7 390	± 1 760	6 695	± 3 004	0.56
Day 90	7 468[b]	± 2 266	7 735[b]	± 4 502	8 674[b]	± 3 554	8 066[b]	± 1 102	0.88

[ab] Means in the same column with different superscripts differ (P<0.05)

No significant differences were found between any of the treatments

Similarly, Mikulski *et al.* (2009) could not find differences in turkeys and Khajali *et al.* (2010) in broilers for the antioxidant capacity between different Se sources. In 2008, Tang *et al.* reported that Se supplementation, but not the source, had an influence on the serum antioxidant capacity of rats, and in contrast with this result, Zong-yun *et al.* (2007) reported that there was a significant difference between organically bound Se and inorganic Se for the antioxidant capacity of cows.

A steady increase was observed for all treatments over the sampling period with a significant effect between day 0 and day 90 ($P < 0.01$) but no effect between the treatments. Very few studies have been publised to date on the effect of Se sources on the TAC of animal plasma, and in most cases different assays were used to determine the TAC, which makes comparisons very difficult and inconclusive. Consistent with the results of earlier studies, no conclusion can be derived from the effect of different Se sources on TAC of wether plasma and further studies on this topic is recommended.

About 11.8% of total selenium in the organism forms part of the GSH-Px complex (Awadeh *et al.,* 1998). Whole blood was taken on a monthly basis from the wethers and the plasma was collected from these blood samples in the laboratory. The plasma was analysed with the Stressgen Kit (Assay Designs Inc., Michigan, USA) and the results are presented in Table 5.2.

The OSA group had a slightly higher value than the other treatment groups after they were randomly assigned at the start of the trial; this may have had some influence on the later results. At day 30 of the trial no significant differences could be established between different Se treatments for the GSH-Px activity in the plasma. However, considering the contrast estimates (Table 5.3) the organically bound Se treatments (OS) produced significantly higher values at day 30 over the CT ($P=0.049$) and IS ($P=0.037$) groups. At day 60 and 90 of the trial no significant differences were observed between treatments and the advantage that the OS group had with the contrast estimates in Table 5.3 was lost ($P=0.429$ and $P= 0.504$). Similarly to the whole blood Se levels, organically bound Se supplementation led to a quicker

response of the GSH-Px levels in plasma, after which an adequate level was reached. Inorganic Se supplementation matched the GSH-Px levels of the OS treatments, but took more time to reach it.

Table 5.2: Plasma GSH-Px activity of Döhne Merino wethers fed diets with different selenium supplements. Mean ± standard error of the mean

	Treatment				
Parameter	CT (u/ml)	IS (u/ml)	OSB (u/ml)	OSA (u/ml)	P-Value
Day 0	11.95 ±2.67	11.38 ±6.03	10.99 ±2.72	14.72 ±3.36	0.77
Day 30	8.68 ±3.64	8.38 ±5.89	12.61 ±4.99	13.69 ±6.42	0.25
Day 60	10.87 ±2.98	10.25 ±2.74	10.99 ±2.72	11.03 ±2.15	0.87
Day 90	13.51 ±5.67	13.69 ±3.89	13.71 ±3.51	16.29 ±4.85	0.87

No significant differences were found between treatments for any of the parameters.

The findings of the present study are consistent with previous ruminant research (Nicholson *et al.*, 1993; Awadeh *et al*., 1998; Knowles, *et al.*, 1999; Malbe *et al*., 1995; Ortman & Pehrson, 1997; Gunter *et al*., 2003) which could not find any significant differences between the GSH-Px activities in the blood of animals that were supplemented with different Se sources. An average increase in GSH-Px activity of 16% was however reported by Weiss (2003) when cattle were fed with organically bound Se.

Significant differences were however reported by Knowles *et al*. (1999) in dairy cows, Pehrson *et al.* (1989) in dairy heifers and Qin *et al.* (2006) who reported that organically bound Se was more effective than Sodium selenite in increasing blood GSH-Px activity in lambs. Furthermore, Rock *et al*. (2001) reported that lambs born to ewes fed with an organically bound Se source had higher concentrations of Se and GSH-Px activity in their blood than lambs born to ewes supplemented with an inorganic Se source.

Table 5.3: P-values of the GSH-Px contrast estimates of the plasma and muscle samples between different treatment groups

	P-values				
Plasma	CT vs OS	IS vs OS	Tissue	CT vs SE	CT vs OSA
Day 30	0.049*	0.037*	Muscle	0.151	0.112
Day 90	0.429	0.504			

*P-value ≤ 0.05

Tissue samples (muscle, liver and kidney) were collected at the abattoir after the wethers were slaughtered at the end of the 90-day trial. The tissue samples were analysed, and the results are presented in Table 5.4. No significant relation between the form of selenium added to the feed and GSH-Px activity in the tissues could be established. Relevant contrast estimates were calculated, as presented in Table 5.3, with no significant contrast in the muscle sample to be reported.

The lack of an effect between different Se supplementations in the tissue samples could possibly be explained by looking back to the whole blood Se levels (Table 4.2) and to the GSH-Px activity in the plasma (Table 5.2). In both these trials there were differences between treatments at day 30, after which these differences disappeared by day 60 and 90. It was concluded that organically bound Se could have a greater effect in the initial supplementation period over an inorganic Se source. However, the differences between sources would disappear over an extended period of time. Therefore no differences were expected between the treatments for the GSH-Px activity in the tissue samples at day 90 when the wethers were slaughtered.

Similar to the present study, Van Ryssen *et al.* (1989) reported no differences between Se sources for the GSH-Px activity in the liver, pancreas or muscle. Also, Juniper *et al.* (2009) could not find differences between sources in the muscle (*Longissimus thoracis)*. Some research groups researched other animal species with Kuricová *et al.* (2003) and Petrovic *et al*. (2006) who could not find a relationship between Se source and GSH-Px activity in the tissue samples of chickens whereas Deagen *et al*. (1986) showed that the Se source could have an influence on GSH-Px activity in the testes and muscle but not in the liver and kidney of rats. Zhan *et al.* (2007) did not observe an effect in the tissue samples of pigs.

Table 5.4: The tissue GSH-Px activity of Döhne Merino wethers fed diets with different selenium supplements. Mean ± standard error of the mean

	Treatment				
Parameter	CT (u/ml)	IS (u/ml)	OSB (u/ml)	OSA (u/ml)	P-Value
Muscle	3.35 ±1.09	4.79 ±3.56	4.09 ±1.62	5.06 ±1.14	0.37
Kidney	12.59 ±4.76	11.15 ±7.25	9.97 ±2.92	12.79 ±4.44	0.65
Liver	13.19 ±8.06	14.29 ±2.89	14.58 ±3.12	15.32 ±4.04	0.86

No significant differences were found between treatments for any of the parameters

An explanation for the lack of any effect between the different Se sources on GSH-Px activity, especially in the tissue samples was based on the fact that all Se compounds must be split into H_2Se before SeCys is synthesised and incorporated into the active centre of the selenoenzymes (Schrauzer, 2000). GSH-Px activity will therefore not be so much dependent on the Se source as on the level of Se supplementation. Another explanation can be that a large amount of the organically bound Se has been directly incorporated into the body protein (Table 4.3 & 4.5), which will have left less Se available for selenoenzymes. This was indeed the case in the present study where higher Se levels were observed in the muscle of the wethers (Chapter 4).

Conclusion

The importance of antioxidant status in human and animal health is well established, with many studies currently focussing on the provision of food with an antioxidant capacity value, or otherwise known as functional food. Numerous diseases are directly linked to oxidative stress of the body. The role of selenium in GSH-Px and its role in preventing oxidation and increasing the antioxidative status of humans and animals are well known and studied. With the increased bioavailability of organically bound Se over inorganic Se now established, this study investigated further advantages of organically bound Se products.

Previous reports on the effect of different Se source supplementation on GSH-Px activity in animals has established a constant trend of numerically higher values for the organically bound Se sources. This could not be confirmed in the present study; the availability of the organically bound Se sources was however observed in the first period of the study. Confirming conclusions made in Chapter 4 that organically bound Se has a higher bioavailability for small ruminants, and supplementation with it will yield a faster response than with inorganic Se supplementation. Over long periods of selenium supplementation there will however not be any differences between Se sources. No effect on GSH-Px activity in the tissue samples between the different Se sources was observed. This can be explained by the route of metabolism and absorption of Se in ruminants which will cause GSH-Px activity to be more dependent on the level of Se supplementation than on its source.

This study has highlighted a few shortcomings in TAC studies and opened the door for further trials. Very few studies have been done to date on the supplementation effect of different Se sources on the TAC of small ruminants and further studies are recommended. In the present study no effect on TAC between the different treatments could be reported, which is consistent with similar trials in other animal species, but varies from a study on cows. The fact that there are numerous different assays to calculate the TAC makes it nearly impossible to make comparisons between studies. Therefore, a standardised assay to calculate TAC is highly recommended.

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CHAPTER 6

The reaction of meat quality (colour, tenderness, cooking loss and drip loss) and lipid oxidation (TBARS) in Döhne Merino wethers fed different selenium source supplementation

Abstract

The objective of this study was to evaluate the quality and lipid oxidation of *M. longissimus dorsi* from wethers supplemented with different selenium (Se) sources. Forty growing Döhne Merino wethers from the Southern Cape region of South Africa, a selenium-deficient area, were used for the study. The animals were all fed the same basal diet in the adaptation period and were then randomly allocated to one of four dietary treatment groups: Control (CT), containing Se from the basal diet only; the NaSe group, fed the basal diet with added Sodium selenite (IS); or one of two groups fed organically bound selenium (Se) in the basal diet with added organically bound Se A (OSA) or B (OSB). The period of supplementation was set at 90 days.

Skeletal muscle samples *M. longissimus dorsi* were collected at day 90 after slaughter to determine their quality. No differences between Se source treatments could be detected in the meat quality (colour, tenderness, drip and cooking loss) after the 90-day supplementation period. Lipid oxidation was measured by determining TBA reactive substances (TBARS) and no differences could be detected. Despite the higher Se levels in the muscles of the organically bound Se groups (see Chapter 4) both Se supplementation and source of Se supplementation failed to have an influence on the lipid oxidation and meat quality of wethers.

Key words: selenium, meat quality, lipid oxidation, TBARS

Introduction

With the discovery of GSH-Px by Rotruck *et al*. (1973) in the early 1970s, a specific biological role for selenium became apparent in the form of selenocysteine, which forms the active centre of this enzyme (Behne & Kyriakopoulos, 2001). The antioxidant functions of Se, via GSH-Px activity, have been shown to persist post-mortem in poultry muscle tissue (DeVore *et al.,* 1983), delaying the onset of oxidation reactions, which adversely affects both the nutritive value and flavour of meat products (Morrissey *et al.,* 1998). A correlation exists between GSH-Px activity and Se content in the tissues of ruminants (Scholz *et al.,* 1981; Gatellier *et al.*, 2004). Therefore the supplementation of diets with Se may increase the oxidative stability of meat.

Almost all meat quality factors are in some way influenced by oxidation, and it has been established that the first line of protection against oxidation is formed by the antioxidant enzymes (Glutathione peroxidase, superoxide dismutase and catalase) (Gatellier *et al*., 2004). It will therefore protect the organism against lipid oxidation and its by-products. Some of these by-products are known to be toxic or carcinogenic while others alter meat flavour negatively (Du & McCormick, 2009). A secondary defence against oxidation can be provided by the natural antioxidants in pastures or feed (Daly *et al*., 1999).

The quality of a potential meat cut purchased is determined by a combination of characteristics that define the level of acceptability for the consumer (Kramer & Twigg, 1962). The visual appearance in the form of colour and in pack purge when the meat is bought; odour and juiciness when the meat is cooked; flavour and tenderness when it is consumed, are some of the characteristics by which the consumer will evaluate the quality of meat (Smith *et al.,* 1970).

The meat must please the eye; therefore meat colour is the main selection parameter which affects the acceptability at the time of purchase (Faustman & Cassens, 1989). Discolouration of the meat surface decreases consumer acceptance (Carpenter *et al.,* 2001) and annually millions are lost in revenue. Nearly 15% of discount retail beef has been reduced in price due to surface discolouration (Smith *et al*. 2000). Meat colour depends on a concentration of pigments (myoglobin, haemoglobin), their chemical states, the type of myoglobin molecule, and the light-scattering properties of meat (Lawrie, 1998). The bright red colour of lamb is due to the oxygenation of myoglobin when meat is exposed to the air. Colour stability is very important, especially in retail display, and can be influenced by several factors, but is mostly linked to oxidation. The strategy to maintain optimum meat colour thus involves the delay of pigment oxidation (Faustman & Cassens, 1989). This can be done with the addition of an antioxidant like rosemary powder to the meat (Sanchez-Escalante *et al*., 2001). As reported by Warren *et al.* (2002) and Wood *et al*. (2004) the formulation of the diet to contain Vitamin E can cause the delay of colour oxidation.

The water-holding capacity (WHC) of meat is defined by Sales (1996) as the ability of meat to retain its water during the application of external factors such as cutting, mincing and storage. Many of the meat quality properties including colour, in pack purge, texture, juiciness and tenderness are only partially dependent on WHC (Lawrie, 1998; Barge *et al*., 1991; Honikel, 1998), while cooking loss and drip loss are directly influenced by WHC (Immonen *et al*., 2000). Normally, muscle post-mortem glycolysis will proceed to an ultimate pH close to the isoelectric point of meat (Lawrie, 1998). This means that there will always be some loss of water post-mortem. Thus, the higher the ultimate pH of muscle, the stronger the binding of water in that muscle (Thomas *et al*., 2004). Therefore, anything that affects the pH and specifically the rate of pH decline, will affect the WHC. This can affect other aspects of meat quality, including juiciness, which plays an important role in the overall palatability of meat.

The consumer considers meat tenderness to be the most important palatability trait (Gonzalez *et al.*, 2001). Meat tenderness is influenced by the myofibrillar component (ultrastructure of the myofibrillar proteins) and the stromal components (content, composition and structure of connective tissue proteins) of the muscle (Muir *et al.,* 1998). Pre-slaughter feeding and animal growth rate have a direct effect on meat tenderness (Fishell *et al.,* 1985) and oxidation might also play a role in controlling the proteolytic activity of enzymes (Starke-Reed & Oliver, 1989) and could be linked to meat tenderness.

Lipid oxidation in meat is one of the most important factors responsible for quality-loss during food storage and production because of the formation of rancid odours and deterioration of flavour (Asghar *et al.,* 1988; Pearson *et al.,* 1977). Any degree of lipid oxidation in raw meat accelerates the development of oxidised off-flavours in cooked meat, due to the free-radical chain-reaction nature of lipid oxidation (Rhee, 1989). The major factors influencing lipid oxidation in raw meat include their fatty-acid composition, endogenous pro-oxidative or anti-oxidative constituents, and non-meat additives (anti-oxidative or pro-oxidative) (Gheisari *et al*., 2010). The degree of lipid oxidation is measured with the thiobarbituric acid reactive substances (TBARS) test, which measures the malonaldehyde (MD) content of the meat, a secondary oxidation product of polyunsaturated fatty acids (Du & McCormick, 2009). It is the most preferred and frequently used test for the determination of lipid oxidation in meat, because of a high and consistent correlation between rancid flavours, aroma scores and TBARS values (Nolan *et al*., 1989).

The objective of this study was to determine if Se supplementation and the type of supplementation (inorganic or organically bound Se) has any influence on the meat quality of small ruminants and its post-mortem oxidative stability.

Materials and methods

The reader is referred to Chapter 3 for the description of the experimental procedures and the treatments used in determining the effect of the different Se supplementations on the performance of the lambs. Table 3.2 is included again for the benefit of the reader regarding the different treatments used in the study.

Table 3.2: Physical and chemical composition of treatment feeds indicating selenium concentration of the experimental diets fed to the Döhne Merino wethers

	CT	IS	OSB	OSA
	Physical composition, As fed basis			
Wheat straw g/kg	760	760	760	760
Maize starch g/kg	150	150	150	150
Molasses meal g/kg	50	50	50	50
Urea g/kg	10	10	10	10
Premix CT g/kg	30	0	0	0
Premix IS g/kg	0	30	0	0
Premix OSB g/kg	0	0	30	0
Premix OSA g/kg	0	0	0	30
	Chemical Composition, DM basis			
Moisture g/kg	95.9	100.9	104.3	105.8
Ash g/kg	50.2	63.7	55	70.6
Crude fat g/kg	6.5	6.9	7	6.9
Crude fibre g/kg	395.8	370.9	315.8	341.2
Crude protein g/kg	84.7	110.7	93.1	125
N g/kg	13.6	16.2	14.9	17.3
Se mg/kg	0.08 (0.14)*	0.27 (0.36)*	0.26 (0.36)*	0.27 (0.32)*

* Values in brackets indicates the actual value as per analysis

Chemical characteristics

Chemical and physical characteristic measurements were conducted on the *M. longissimus dorsi* (LD) after it had been excised in the abattoir from the carcasses' left side from between the 8th and 11th thoracic vertebrae, according to descriptions by Honikel (1998). Before any physical analyses were carried out, all visible fat was trimmed from the muscles.

Lipid oxidation was measured by the thiobarbituric acid reactive substances (TBARS) method as proposed by Lynch & Frei (1993) and adapted and modified by Gattellier *et al.* (2001); Rosmini *et al.* (1996) and Fernandez-Lopez *et al*. (2007). Muscle samples (LD*)* of 1g each were homogenised in 10ml of a prepared mix (5.6g KCl + 0.01g BHT and 500ml distilled water) with a Polytron homogenizer (1 minute, medium speed). Samples of 0.5ml homogenate were incubated with 1% (w/v) 2-thiobarbituric acid in 50mM NaOH (0.25ml) and 2.8% (w/v) trichloroacetic acid (0.25ml) in a boiling water bath for 10 minutes. After cooling to room temperature under running tap water, 2ml n-Butanol was added and placed in a vortex to extract the pink chromogen, and its absorbance was measured at 53nm against a blank of n-Butanol. TBARS concentrations were calculated using 1,1,3,3 tetraethoxypropane (0-0.8µM) as standard. Results were expressed as TBARS in mg malondialdehyde (MDA) per kg muscle.

Physical characteristics

For the colour measurements of the muscle, freshly cut slices (1.5–2.0cm thick) were allowed to bloom for thirty minutes at room temperature (18-19 °C). After the colour was recorded in triplicate, in positions selected at random on the slice surface (Stevenson *et al*., 1989) the colour was expressed by the coordinates L*, a* and b* of the CIELab colorimetric space (Commision Internationale de l'Eclairage, 1976) with the use of a Colour guide $45^0/0^0$ colorimeter (BYK-Gardner, USA). The L* represents lightness in meat colour, a* indicates red-green range and b* the yellow-blue range (Poulsen *et al*., 2004).

Weighed muscle slices (1.5–2.0cm thick), cut perpendicular to the longitudinal axis of the muscle on the caudal side of the removed LD were used to determine drip loss. The slices were suspended in inflated plastic bags, ensuring that the slices did not come into contact with the bag, and left in a refrigerator (4^0C) for 24 hours. After the storage period the samples were blotted dry with absorbent paper and weighed again. The drip loss was expressed as a percentage of the initial weight of the sample (Honikel, 1998).

Cooking loss of the LD was determined by placing freshly cut, weighed samples (1.5–2.0cm thick), in thin-walled sealed plastic bags, and in a preheated water bath (80 °C) for 1 hour (Honikel, 1998). The cooked meat samples were then removed from the water bath and allowed to cool under running water. Excess water was blotted with absorbent paper before the weight was recorded. Cooking loss was calculated as the difference in sample weight before and after cooking, expressed as a percentage of the initial weight of the sample.

The same muscle samples that were used to determine cooking loss were used for the assessment of tenderness. Muscle samples were stored overnight (4 $^{\circ}$C) and care was taken to ensure that no visible connective tissue was included in the sample before tenderness was determined. The tenderness assessment was performed as described by Wheeler *et al*. (2001) and Honikel (1998), by using a Warner-Bratzler device, with a load cell of 2.000kN, attached to the Model 4444 Instron texture machine (Apollo Scientific cc, South Africa). Three core samples were cut perpendicular to the longitudinal axis of the muscle fibres so that the influence of the myofibrillar proteins on the shear force could be measured (Voisey, 1976). These samples were placed in the Warner-Bratzler device, so that the knife blade of the device cut across the fibres at a right angle. Mean shear force values were calculated from the recorded shear force values for three cylindrical cores from each muscle sample and used in the statistical analysis. A higher value indicated greater shear force and therefore, tougher meat (Honikel, 1998).

Statistical analysis

Repeated measures ANOVA were made of treatments over a number of days to measure if interactions between treatments and days were significant, and multiple comparisons were made of the interaction effects. If interactions were not significant, main effects were similarly analysed. When ANOVAs were made of the appropriate response variable among the treatments, the treatment effects were analysed

with Bonferroni multiple comparisons (if significant) with appropriate contrast among the treatment effects (Statistica version 8.1 (2008)). Differences were considered significant when P<0.05.

Results and discussion

The TBARS values essentially represent the shelf-life of the meat post-mortem. Analysis was done on days 0, 6 and 12 after slaughter and the results are presented in Table 6.1. No Se supplementation or source influenced the results and no significant differences were detected.

Table 6.1: TBARS values (mg MDA/kg meat) over a 12-day period of muscle samples from wethers supplemented with different selenium sources. Mean ± standard error of the mean

	Treatment				
Parameter	CT	IS	OSB	OSA	P-value
Day 0	2.94 ± 0.4	2.93 ± 0.4	2.03 ± 0.5	2.46 ± 0.4	0.75
Day 6	3.61 ± 1.3	5.37 ± 1.3	3.2 ± 1.5	4.89 ± 1.1	0.41
Day 12	3.65 ± 1.1	3.82 ± 1.1	3.12 ± 1.3	4.25 ± 1.1	0.30

No significant differences were found between treatments for any of the parameters

These results are consistent with previous reports by Vignola *et al.* (2009) and Juniper *et al.* (2009) who could not find any significant differences in the muscle TBARS values of the lambs supplemented with different Se sources. The lack of any effect on the oxidative stability (TBARS) of the *M. longissimus thoracis* (LT) in calves was reported in a similar investigation of different types of Se supplementation by Skřivanová *et al.* (2007).

Juniper *et al*. (2008) also reported no statistical differences in TBARS values for LM among Se treatments in beef cattle. Taylor *et al.* (2008) confirmed these results, reporting that the shelf-life of steaks from Se supplemented cattle was the same as those from cattle which had not undergone supplementation.

In this present study a number of parameters whereby meat quality is defined (cooking loss, drip loss, tenderness and colour) were analysed. The possible influences on these parameters by supplementing small ruminants with different sources of Se were determined. The results (Table 6.2) indicated no significant effects and that no relationship between Se source and meat quality could be established.

Similar results were reported by Marounek *et al*. (2006) who found that Se yeast supplementation for veal calves did not influence these quality parameters. No significant differences in these meat quality parameters could be found by Vignola *et al.* (2009) when lambs were supplemented with different Se sources. Skřivanova *et al.* (2007) fed calves with different Se supplement sources but could not establish different effects between sources of supplementation on muscle drip-loss or colour.

Table 6.2: Meat quality parameters of Döhne Merino wethers fed diets with different selenium treatments. Mean ± standard error of the mean

	Treatment				
Parameter	CT	IS	OSB	OSA	P-value
Cooking loss %	30.15 ± 2.6	31.69 ± 3.5	31.18 ± 2.3	32.37 ± 2.6	0.35
Drip Loss %	2.13 ± 0.3	2.2 ± 0.6	2.18 ± 0.4	2.3 ± 0.4	0.84
Tenderness (N)	3.26 ± 0.3	3.16 ± 0.4	3.01 ± 0.3	3.06 ± 0.6	0.58
Colour L*	36.43 ± 3.7	37.7 ± 3.7	39.99 ± 2.7	39.48 ± 6.1	0.23
a*	13.76 ± 1.5	13.2 ± 1.1	12.87 ± 0.8	13.37 ± 1.9	0.57
b*	10.09 ± 1.7	9.89 ± 2.1	10.75 ± 1.2	10.9 ± 1.7	0.44

No significant differences were found between treatments for any of the parameters

Conversely, Zhan *et al.* (2007) concluded that dietary SeMet contributed to a significant reduction in drip loss from the loin muscle in finishing pigs and that it also played a role in meat colour. However, Mahan *et al.* (1999) and Mateo *et al.* (2007) reported a higher numerical drip loss in pig meat when the pigs were fed diets supplemented with inorganic sodium selenite than with an organically bound Se source. The Se source had a non-significant influence on the colour as well. A number of trials looked at drip loss in broiler meat and suggested a positive effect of Se-yeast over inorganic or conventional Se supplementation (Edens, 1996; Naylor *et al*., 2000; Downs *et al.,* 1999). Dunshea *et al*. (2005) found that organic forms of Se might have a beneficial impact on meat quality, related to its effects on drip loss.

It therefore appears that different Se sources (the organically bound Se providing the greatest advantage) will have an influence on the meat quality of monogastric animals, but not in small ruminants as observed in this study.

Conclusions

An argument was postulated that organically bound Se, which has been proven to be more bioavailable than it's inorganic or conventional counterpart, should have a positive influence on lipid oxidation and meat quality. This was however proven incorrect in the present study and confirmed by numerous other researchers who found similar results. This was expected after it was discovered (see Chapter 5) that the Se source did not influence the activity of the GSH-Px in the muscle samples.

It can therefore be concluded that the Se source will not influence meat quality or lipid oxidation in Dohne Merino sheep if there is no difference between the GSH-Px activities in the meat. This strengthens the argument in Chapter 5 that because of the route of absorption and metabolism of Se in ruminants, the Se source will not have an influence on GSH-Px activity. Therefore, the ability of the Se source to improve the meat quality and self-life of meat is very limited.

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CHAPTER 7

General conclusion

In South Africa, thousands of people are directly dependent on sheep farming for their food and livelihood, with millions dependent in turn on livestock farmers to provide them with sufficient good quality protein.

The specific objectives of this research were threefold: to compare the effects of inorganic and organically bound selenium sources on small ruminant performance, to investigate the deposition of these selenium sources in various tissue types and wool and to analyse their influence on carcass characteristics, meat quality and antioxidant capabilities.

New technologies and better farming systems are required to meet the growing demand for protein. South Africa is no different from the rest of the world and the soil is becoming depleted of Se, leading to selenium-poor plants, animals and therefore humans. Selenium is recognised as an essential trace element for the maintenance of health, growth and a myriad of biochemical-physiological functions. In recent years the importance of adequate Se levels to maintain human and animal health has become more evident.

This project was conducted to obtain more information on the differences between inorganic and organically bound Se sources and to assess their effects and advantages on small ruminants. This was done by supplementing a group of Döhne Merino wethers with different Se sources in a controlled and measurable intake. A number of different samples (blood, wool and tissue) were collected during the set period of 90 days of the study. These samples were analysed with a variety of kits and protocols (production parameters, deposition levels, GSH-PX, ORAC, TBARS and meat quality) to find the differences between organically bound and inorganic Se supplementation for Döhne Merino wethers.

Results obtained from the production and performance of the sheep did not produce any differences between the different Se sources. Selenium levels in the wethers were only marginally deficient at the start of the trial; some Se was available to all groups from the basal diet and this may have been sufficient to meet the basic Se requirements of the lambs. They had also passed their optimal growth phase after the adaptation period. These could be some of the alternative reasons behind the insignificant difference in results between the various Se sources. Therefore, a possible difference in effect between Se sources cannot be ruled out, under different environment and conditions.

The results from the deposition level (bioavailability) study produced interesting results. In the whole blood Se study it was found that organically bound Se was more readily available to the wethers and would therefore be the preferable choice to rectify evident Se deficiency in small ruminants. However, when Se was supplemented for extended periods, there was no difference between the sources

according to the whole blood Se levels. The Se source used to supplement small ruminants for extended periods can therefore be the consumer's choice.

Significant differences ($P<0.05$) were observed between the deposition levels of the Se sources in the analysis of the muscle, kidney and wool samples. The organically bound Se sources were proven to have a greater incorporation into the tissue samples than the inorganic Se source. This is mainly due to the different absorption paths of the two Se sources whereby the organically bound sources can be absorbed through amino-acid transport and directly incorporated into body protein. Small ruminants which are supplemented with organically bound Se sources will therefore have the advantage of accumulated Se reserves in the body. This Se reserve could be available in stress conditions when the requirement for Se is high, but needs further investigation. The Se accumulation in the body protein will also hold benefits for the consumers of these animal products, with more natural Se available in their diets.

Results for the Se deposition in the liver were inconsistent with previous research reports. No significant ($P>0.05$) differences were observed between the Se sources. The only difference between this study and previous reports was in the blood Se levels recorded at the end of the trial, just before tissue collection. Therefore, the Se levels in the liver are probably more dependent on the Se levels present in the blood of the small ruminant, than on the supplemented form used in the diet.

Selenium forms part of the GSH-Px enzyme, which plays an important role in human and animal health as a natural antioxidant. Similar results were recorded with the GSH-Px activity in the plasma as with the Se levels in the whole blood, with significant ($P<0.05$) contrast recorded in the early stages (first 30 days) of the study but no differences between sources at the end of the study. With no differences between the GSH-Px activities in the plasma at the end of the trial, the results from the tissue samples were only to be expected. No differences between the Se sources could be detected in the GSH-Px activity in the tissue samples. These results confirmed that organically bound Se is more readily available to small ruminants than inorganic Se, but that there are no differences between Se sources if it is supplemented for extended periods of time.

Regarding the TAC of the plasma from the supplemented animals, which was measured with the ORAC assay, no differences between treatments could be observed. This is consistent with the few previous TAC studies on different animal species. Very few studies have been done to date on the supplementation effects of different Se sources on the TAC of small ruminants, and further studies are recommended. The fact that there are numerous different assays to calculate the TAC makes it nearly impossible to make proper comparisons between studies. Therefore, a standardised assay to calculate TAC is also highly recommended.

Since no differences were reported between Se sources in the GSH-Px activity in the muscle samples, no differences were expected in the lipid oxidation study, and this was confirmed by the TBARS analysis. The Se source did not influence the shelf-life of the meat and no differences occurred between the Se

sources for any of the meat quality parameters (cooking loss, drip loss, tenderness and colour), which was consistent with a number of previous reports. It was concluded that if there are no differences in GSH-Px activity in the meat between treatment groups, the Se source will not influence the meat quality or lipid oxidation of meat in ruminants. This strengthens the argument that because of the route of absorption and metabolism of Se in ruminants, the Se source will not have an influence on GSH-Px activity. Therefore, the Se source has limited potential for improving the quality of meat or its shelf-life.

Based on the results obtained in this investigation, it may be inferred that organically bound Se (OSA & OSB) supplementation will hold a number of advantages for small ruminants over inorganic Se supplementation. Organically bound Se has a greater bioavailability than does inorganic Se, as it is better absorbed and assimilated into body protein. It will therefore have a positive impact on small ruminant health and production, which will result in an indirect advantage for consumer health. Are these advantages of organically bound Se cost-effective? What is the economic impact of changing from an inorganic Se source to an organically bound Se source for supplementation? These are questions that need to be answered in further studies.